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# Glutathione, why we need it every day?

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**Abbreviation:**

| | |
|---|---|
| ABEI: | N-(4-aminobutyl)-N-ethylisoluminol |
| CDP | Cytidine diphosphate |
| dCDP | Deoxy cytidine diphosphate |
| GSH | Glutathione |
| GSSG | Glutathione disulfide |
| HPLC | high-performance liquid chromatography |
| OPA | o-phthalaldehyde |
| -SH | sulfhydryl group |

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The tripeptide thiol glutathione (GSH) has facile electron-donating capacity, linked to its sulfhydryl (-SH) group. Glutathione is an important water-phase antioxidant and essential cofactor for antioxidant enzymes. Its high electron-donating capacity is used to regulate a complex thiol-exchange system. While considering antioxidant, glutathione-S-transferases and glutathione peroxidase are two important enzymes involved in. This review covers the chemistry aspects of GSH as an electron donor as well as a hydrogen donor. The suitable method for the simultaneous determination of thiols is also mentioned.

**I**

The earliest record about glutathione comes back to 1890 by de Rey-Pailhade [1]. He named it philothion (from the Greek words for love and sulfur). Today, we know that glutathione is composed of three amino acids --- γGlu-Cys-Gly. That compound was first synthesized by duVigneaud and Miller in 1936 [1]. Glutathione is a key substance found in every cell in our body, and may be thought of as a "naturally occurring universal antioxidant" – and one without adverse side effects! It is one of the cell's most important antioxidants, neutralizing "free radicals" that would otherwise damage or destroy the cells [2]. Glutathione is also a very important detoxifying agent, enabling the body to get rid of undesirable toxins and pollutants. It forms a soluble compound with the toxin that can then be excreted through the urine or the gut [3]. It is required in many of the intricate steps needed to carry out an immune response [3]. Glutathione values decline with age and higher values in older people are seen to correlate with better health, underscoring the importance of this remarkable substance for maintaining a healthy, well-functioning body [3]. The importance of glutathione cannot be overstated. That's the reason why so many researchers are working hard on it. This mini review will focus on the biochemistry of glutathione, such as the molecular characteristics, key enzymes and typical reactions.

**Molecular characteristics:**

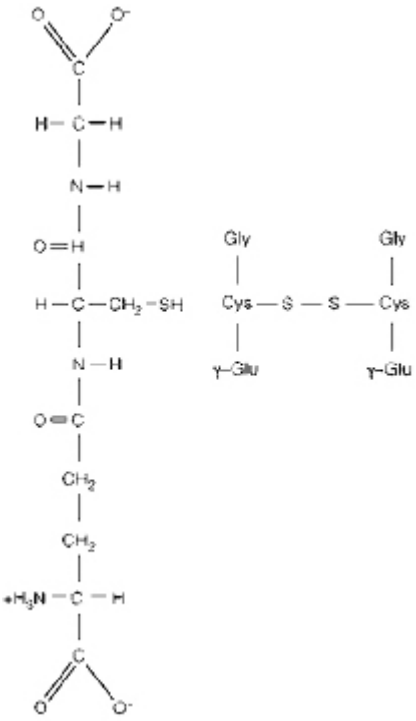


**Figure 1**. Structure of glutathione (left) and glutathione disulfide (right) [5].

The tripeptide thiol glutathione (GSH) has facile electron-donating capacity, linked to its sulfhydryl (-SH) group. It can be written as $C_{10}H_{17}N_3O_6S$ (molecular weight 307.3 Da). Glutathione exists in two forms (Fig. 1) [5]: The antioxidant of called "reduced glutathione" is a tripeptide conventionally called glutathione and abbreviated GSH; one oxidized form is a sulfur-sulfur linked compound, known as glutathione disulfide or GSSG. The GSSG/GSH ratio may be a sensitive indicator of oxidative stress [4]. The strong antioxidant ability is due to its facile electron-donating capacity. This capacity is indicated by the high negative redox potential of the GSH/GSSH "redox couple" ($E^{o'}$ =-240mV, pH7.0; $E^{o'}$ =-267mV, pH7.4 ) [5].

**Source of glutathione:**

GSH cannot enter most cells directly and therefore must be made inside the cell, from its three constituent amino acids: glycine, glutamate and cysteine. The rate at which glutathione can be made depends on the availability of cysteine, which is relatively scarce in foodstuffs. Furthermore, it is the cysteine molecule that has the sulfur-containing portion that gives the whole glutathione molecule its 'biochemical activity' [3]. Cysteine is

generated from the essential amino acid methionine, from the degradation of dietary protein, or from turnover of endogenous proteins. The overall GSH metabolism is summarized by way of the γ-glutamyl cycle in Figure 2 [6].

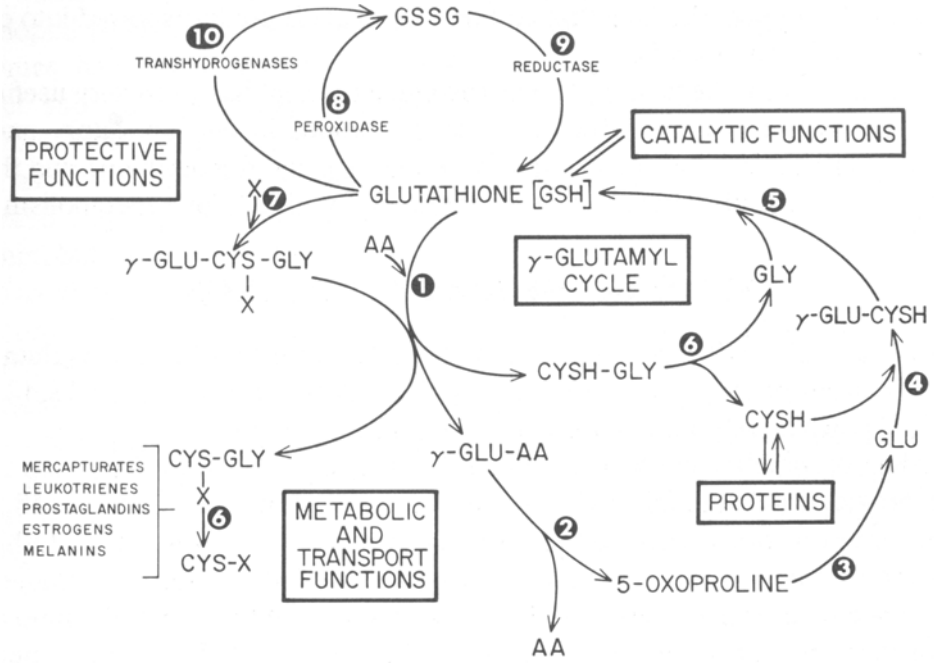


**Figure 2.** Overall GSH metabolism. 1.γ-glutamy-transpeptidase; 2.γ-glutamycyclotransferase; 3. 5-oxoprolinase; 4. γ-glutamycysteine syn-thetase; 5.glutathione synthetase;6.dipeptidase; 7. glutathione S-trans-ferase; 8. glutathione peroxidase; 9. glutathione reductase; 10. transhydrogenases. **AA**=amino acids; **X**=compounds react with GSH to yield conjugates.

(Adapted from [6]).

The key enzymes involved in these reactions are: 1, cysteine and glutamate are combined (by the enzyme γ-glutamylcysteine synthetase); 2, GSH synthetase catalyses combining γ-glutamylcysteine with glycine to generate GSH. Figure 3 shows the model of reaction catalyzed by γ-glutamylcysteine synthetase [7]. The figure shows that cysteine and γ-Glu are put close to form the GSH while the SH group is protected by the enzyme. In the other hand,

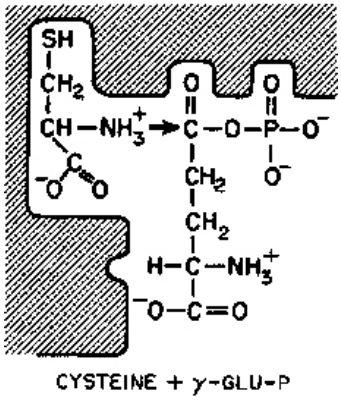


**Figure 3.** Active center in γ-glutamyl-cysteine synthetase. (Adapted from [7])

if some drugs with the similar shape fit into the active site of the enzyme, it will inhibit the synthesis of GSH [7].

**Chemical characteristics and typical reactions:**

The GSH pool is drawn on for three major applications: (**a**) as cofactor for the GSG-S-transferases in the detoxicative pathways (Reaction 7 in Fig. 2); (**b**) as substrate for the γ-glutamyl transpeptidases, enzymes which are located on the outer cell surface and which transfer the glutamine moiety from GSH to other amino acids for subsequent uptake into the cell (Reaction 1 in Fig. 2); and (**c**) for direct free-radical scavenging and as an antioxidant enzyme cofactor (Reaction 8 in Fig. 2) [6]. I can't include all aspects of chemistry characteristic about GSH here, but (a) and (c) are the two important pathways that relate to detoxication.

The preferred ligands recognized by the glutathione S-transferases are predominantly hydrophobic [8]. Glutathione S-transferase activate attack by GSH on an electrophilic center. The most common groups have such sites including alkyl halides (1), lactones (2), epoxides (3), sulphates (4), α, β-unsaturated compounds (5), quinones and quinonimines (6), esters (7), arylhalides (8) and aryl nitro compounds (9) (Figure 4) [8].

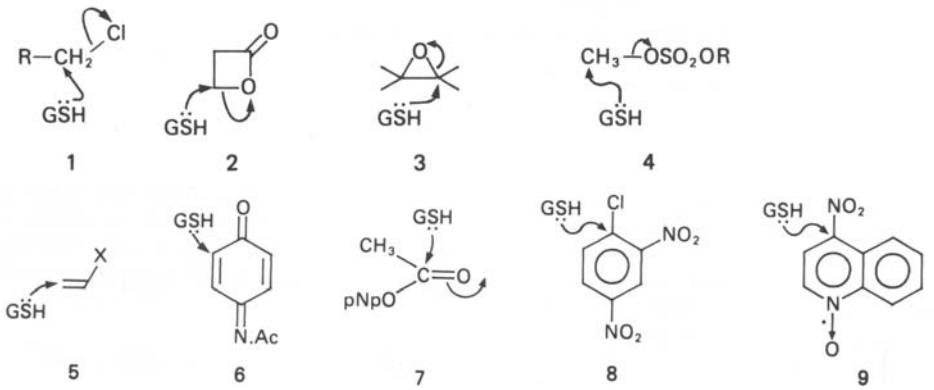

**Figure 4.** Nine kinds of electrophilic carbon site in hydrophobic compounds which react with GSH (Adapted from [8]).

Besides donating electron, GSH can also donate hydrogen while reacts with

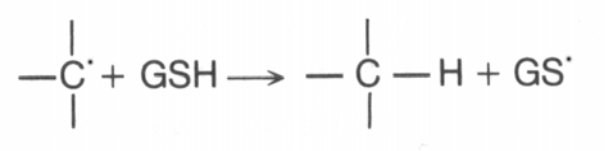


**Figure 5**. Formation of the glutathione thiyl radical. Adapted from [9]

free radicals (Figure 5) [9]. The rate constants of the reaction depend on the kind of radicals. Table 1 is the values of the rate constants for some solution at room temperature [9]. The hydrogen donation reaction is energetically favourable because the C-H bond strength is higher than that of the S-H bond [9]. The hydrogen donation reactions can be very rapid.

**Table 1:** Rate constants for production of GS˙ by carbon-centred radicals [9].

| Radical | $10^{-7}$ $k_1$ ($dm^3$ $mol^{-1}$ $s^{-1}$) |
|---|---|
| Methanol | 4.0 |
| Ethanol | 11 |
| 2-Propanol | 18 |
| Glucose | 0.7 |
| Deoxyribose | 3.5 |

Because the concentrations of radicals is much less than that of the thiol, the reacton in Fig. 5 can be termed “pseudo first order” [9]. The decay of the carbon-centred radicals (equal to the rate of formation of GS˙) will be very close to exponential, with half-life given by: $t_{1/2}$=In $2/(k_1[GSH])$ [9]. Thus with typical GSH concentration (1-2 mmol $dm^{-3}$) and $k_1$=5 X $10^7$ $dm^3$ $mol^{-1}$ $s^{-1}$, the $t_{1/2}$ of the yielded thiyl radicals is about tens of microseconds [9]. GS˙ disappears by conjugating to other reactants with a high speed [9].

$GS^{\cdot} + GS^- \overset{k_1}{\rightleftharpoons} GSSG^{\cdot -}$ ($k_1$= 6 X $10^8$ $dm^3$ $mol^{-1}$ $s^{-1}$; $k_2$ = 2 X $10^5$ $dm^3$ $mol^{-1}$ $s^{-1}$)

$$GS^{\cdot} + O_2 \xrightarrow{k_2} GSOO^{\cdot} \quad (k = 2 \times 10^9\ dm^3\ mol^{-1}\ s^{-1})$$

$$GS^{\cdot} + NADH \longrightarrow GS^- + NAD^{\cdot} + H^+ \quad (k = 10^4\ dm^3\ mol^{-1}\ s^{-1})$$

$$GS^{\cdot} + GS^{\cdot} \longrightarrow GSSG \quad (k = 9 \times 10^8\ dm^3\ mol^{-1}\ s^{-1})$$

The last reaction is part of those in Figure 2 reaction 9, and it's really fast. In our body, the major GSSG is produced by glutathione peroxidase. After GSH has been oxidized to GSSG, the recycling of GSSG to GSH is accomplished mainly by the enzyme glutathione reductase. This enzyme uses as its source of electrons the coenzyme NADPH (nicotinamide adenine dinucleotide phosphate, reduced). Therefore NADPH, coming mainly from the pentose phosphate shunt, is the predominant source of GSH reducing power.

GSH is also involved in DNA synthesis. Ribonucleotide reductase and glutaredoxin catalyze the first step in DNA synthesis by reducing the four different ribonucleotides to the corresponding deoxyribonucleotides. GSH is the hydrogen donor for this reaction. After that, GSSG will be reduced to GSH [10].

$$CDP + 2\ GSH \xrightarrow[\text{Glutaredoxin}]{\text{Ribonucleotide reductase}} dCDP + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{\text{Glutathione reductase}} 2\ GSH + NADP^+$$

**Detection of GSH:**

The suitable method for the simultaneous determination of thiols is high-performance liquid chromatography (HPLC). The method is based on

the precolumn derivation of thiols with o-phthalaldehyde and N-(4-aminobutyl)-N-ethylisoluminol to form isoindole derivatives and the reversed-phase HPLC separation of the derivation, followed by successive

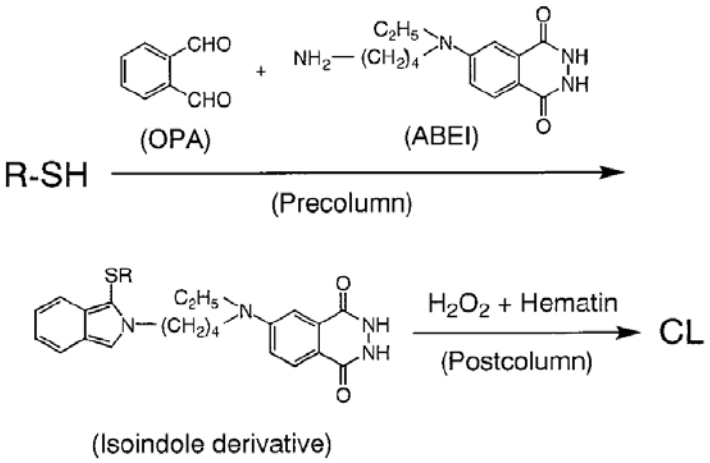


**Figure 6**. Precolumn and postcolumn derivation of thiols for their on-line chemiluminescence determination.
**CL**= chemiluminescence.
(adapted from [11])

postcolumn chemiluminescence reactions with hydrogen peroxide and hematin (Figure 6)[11].

The typical result is showed in Figure 7 [11].

**Summary:**

GSH is an important water-phase antioxidant and essential cofactor for antioxidant enzymes. Its high electron-donating capacity dues to its sulfhydryl (-SH) group. It also can be a hydrogen donator when react with free radicals. We need GSH everyday to be an antioxidant, to maintain

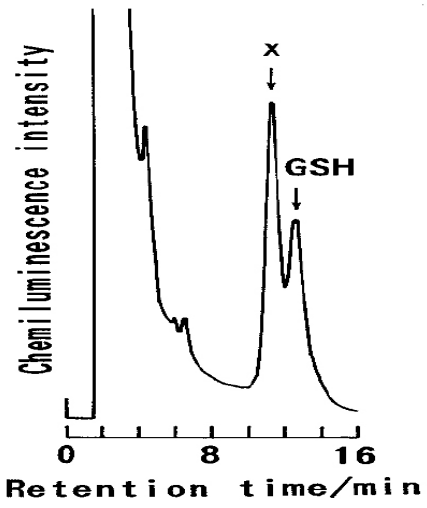


**Figure 7**. HPLC result of determination of precolumn derivation product of GSH. X represents a peak suspected to be the Schiff base formed from OPA and ABEI. (adapted from [11])

metabolism, to slow aging even to neutralize carcinogenic chemicals.

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