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COMMENTARY

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Measurement and Protective Functions of Glutathione

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Description

Glutathione is essential for the proper functioning of the immune system and for tissue building and repair. Glutathione is a substance made from the amino acids glycine, cysteine, and glutamic acid. It is produced by the liver and is involved in many body processes. To measure total GSH, a recycling assay is used, in which GSH reacts with the conjugate to produce GSSG and another TNB molecule, which can increase fluorescence or absorbance. The enzyme glutathione reductase then reduces the Glutathione disulphide, releasing the GSH that can react with another DTNB molecule.

Protective functions

Reduction: In people who smoke or inhale particulate matter or other oxidants, there is a potential for inflammation, in which neutrophils enter the airspaces from the blood through the endothelial and epithelial cells. When these neutrophils squeeze between the cells, they release Hypochlorous acid, which can react with GSH, which is secreted by the epithelial cells and normally protects the epithelial cells. In cystic fibrosis patients, who secrete less GSH than normal individuals into the lining fluid covering their alveoli, and in smokers who have exposed their lungs to many oxidants including nitrogen dioxide and hydrogen peroxide, there is both chronic inflammation and lower GSH than normal.

Conjugation: Elimination of many xenobiotic compounds can be achieved by conjugation with GSH, followed by secretion of the adduct from the cell. Although the Quinone, menadione can react with GSH to form an adduct non-enzymatically, an enzymatically catalyzed Michael addition by a Glutathione S-Transferase (GST) is much faster. The glutathione adduct can then be secreted from cells through a membrane transporter such as the multidrug resistant proteins.

Interaction with other non-enzymatic antioxidants

While GSH is the main low molecular weight antioxidant produced in cells, there are other low molecular weight antioxidants that are obtained from food, such as vitamins E (α-tocopherol) and C (ascorbic acid). Vitamin E can reduce lipid hydroxyl radicals and lipid peroxides generated from polyunsaturated fatty acids. The oxidized vitamin E is then reduced by vitamin C in a non-enzymatic but rapid reaction. The oxidized vitamin C can then be converted back to the reduced form by enzymatic reactions, one of which uses GSH as a substrate.

Measurement of glutathione

One of the most important problems in determining the mechanisms of both oxidative stress and redox signaling is the measurement of the different forms of thiols in cells. The predominant forms are the reduced form of GSH and GSSG. Nitrosoglutathione (GSNO) and Protein Nitrosothiols (PSNO) are also formed in cells and play a role in NO signaling independent of the cyclic GMP pathway. Cysteine is a precursor amino acid to GSH and cysteine is the disulphide form of cysteine. Protein thiols exist as cysteine, mixed disulphides between cysteine and GSH or other thiols, and disulphides between two protein cysteine's, which may be present in the same or different protein molecules. It is important to recognize that an increase in the oxidized forms of these thiols in the cytosol is transient even during oxidative stress. Therefore, it can be very difficult to measure thiol oxidation, especially that occurring in signal transduction. GSH is modified with N-Ethyl Maleimide (NEM) or vinyl pyridine. To measure mixed protein disulphides, the GSH can be released from the mixed protein disulphide with sodium borohydride ($NaBH_4$) and the GSH then measured in the recycling assay.

A more commonly used method to measure GSH and GSSG is now High Performance Liquid Chromatography (HPLC).

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In this assay, thiol compounds are first modified by the addition of iodoacetate. The amino groups of the compound are then modified with 1-fluoro-2, 4-dinitrobenzene. This then allows for the separation of many compounds that can be identified by their movement on HPLC.