Sem – II (PG)

Paper ZOO-202

Group B: Biochemistry

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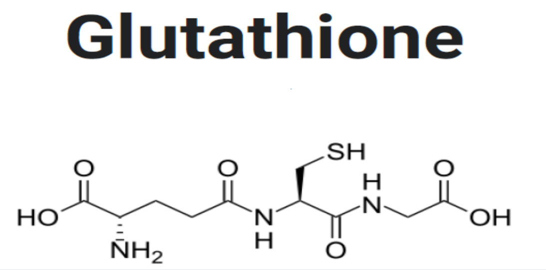
Protein metabolism

Formation of specialized products from amino acids

Glutathione

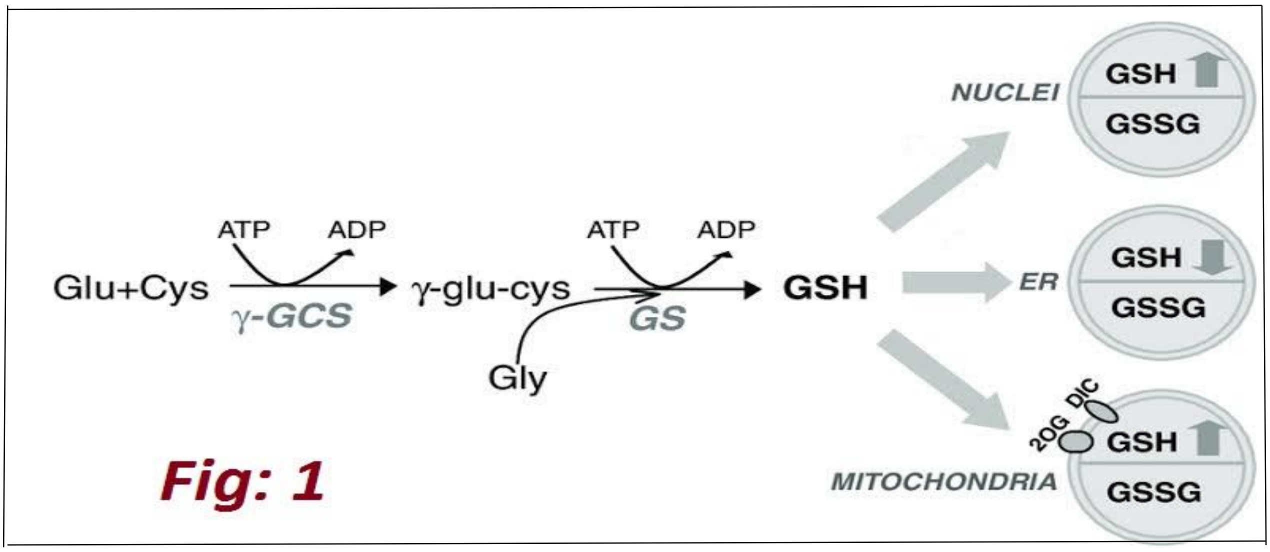
Glutathione / γ-glutamylcysteinyl glycine (GSH) is an antioxidant in plants, animals, fungi, some bacteria and archaea. Glutathione is capable of preventing damage to important cellular components caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals. It is a tripeptide with a gamma peptide linkage between the carboxyl group of the glutamate side chain and cysteine. The carboxyl group of the cysteine residue is attached by normal peptide linkage to glycine.

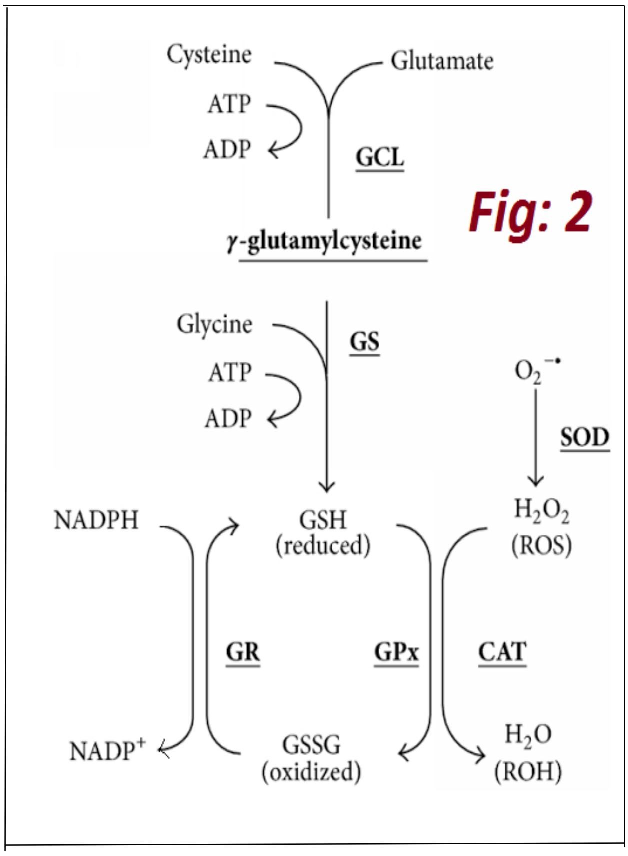


Biosynthesis:

Glutathione biosynthesis involves two adenosine triphosphate-dependent steps:

- First, gamma-glutamylcysteine is synthesized from L-glutamate and cysteine. This conversion requires the enzyme glutamate–cysteine ligase (GCL)/ glutamate cysteine synthase (GCS). This reaction is the rate-limiting step in glutathione synthesis.
- Second, glycine is added to the C-terminal of gamma-glutamylcysteine. This condensation is catalyzed by glutathione synthetase (GS).





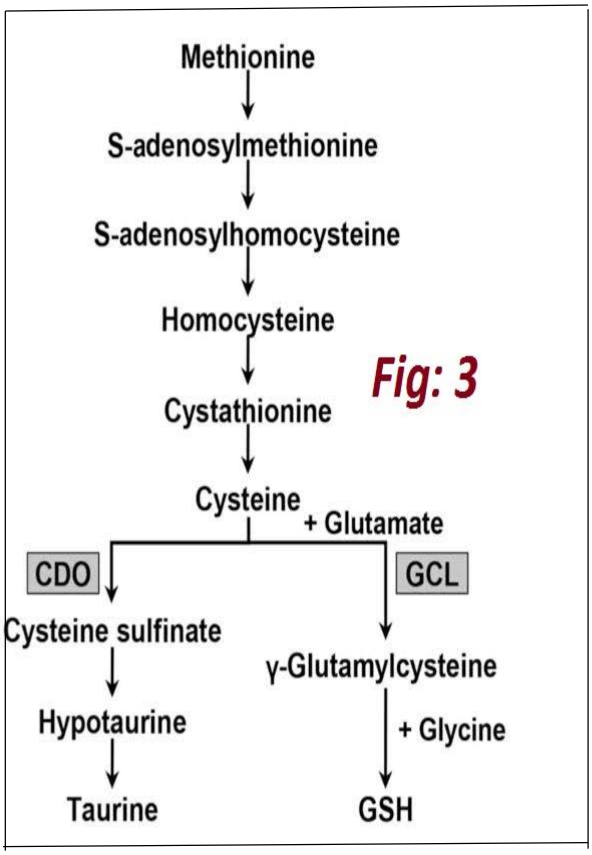


Fig 1: Glutamine synthesis pathway with ratio of reduced & oxidized state in different organelles.

Fig 2: Pathway shown with cofactor NADPH/NADP$^+$ and antioxidant property (ROS). GR requires FAD, GP$_x$ requires selenium.

Fig 3: Although cysteine itself is an amino acid, it may also come from another amino acid methionine. Right side is the pathway for glutathione synthesis, another side is for another product.

GR: Glutathione Reductase, GP$_x$: Glutathione Peroxidase, SOD: Superoxide Dismutase, CAT: Catalase.

Glutathione exists in reduced (GSH) and oxidized (GSSG) states. The ratio of reduced glutathione to oxidized glutathione within cells is a measure of cellular oxidative stress where increased GSSG-to-GSH ratio is indicative of greater oxidative stress. In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH), with the remainder in the disulphide form, namely glutathione disulphide (GSSG) (Fig 1).

In the reduced state, the thiol group of cysteinyl residue is a source of one reducing equivalent, Glutathione disulphide (GSSG) is thereby generated. The oxidized state is converted to the reduced state by NADPH. This conversion is catalyzed by glutathione reductase (GR):

$NADPH + GSSG + H_2O \rightarrow 2\ GSH + NADP^+ + OH^-$

GSH protects cells by neutralising (i.e., reducing) reactive oxygen species into water molecule (H_2O). This conversion is illustrated by the reduction of peroxides:

$2\ GSH + R_2O_2 = GSSG + 2\ ROH$

And with free radicals:

$GSH + R^{\cdot} = 0.5\ GSSG + RH$

Location:

Glutathione is the most abundant thiol in animal cells. It is present both in the cytosol and the organelles. Humans synthesize glutathione, but a few eukaryotes do not, including Fabaceae, *Entamoeba*, and *Giardia*. The only archaea that make glutathione are halobacteria. Some bacteria, such as cyanobacteria and proteobacteria, can biosynthesize glutathione.

Functions:

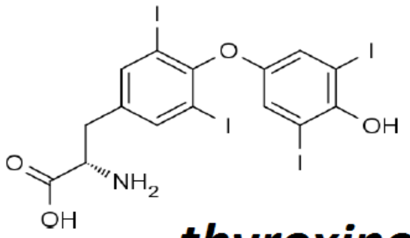
GSH plays important roles in i) free radicals scavengers (antioxidant), ii) biosynthesis of leukotrienes & prostaglandins, iii) metabolism of xenobiotics, iv) neuromodulation (act as neurotransmitter), v) plant defence system.

Glutathione and human diseases:

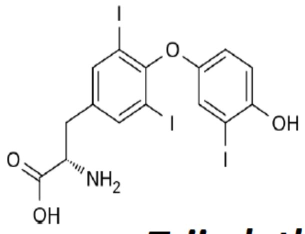
GSH is related to some diseases like cancer, Alzheimer’s disease, cystic fibrosis etc. In these cases, ROS produced after therapy, neutralized by high levels of GSH.

<u>*Thyroid hormones (T_3 and T_4)*</u>

Thyroid hormones are amine hormones and so their synthesis is based on the amino acid tyrosine. The primary synthetic organ of Thyroid Hormones is the thyroid gland which produces about 20 times more T_4 compared to T_3. T_4 is then converted to T_3 by Iodinase enzyme which is present throughout the body's tissues.



thyroxine (T4)



Triiodothyronine (T3)

Biosynthesis:

Thyroid hormones (T4 and T3) are produced by the follicular cells of the thyroid gland and are regulated by TSH made by the thyrotropes of the anterior pituitary gland. Thyroxine (3, 5, 3′,5′-tetraiodothyronine) is produced by follicular cells of the thyroid gland. It is produced as the precursor thyroglobulin (this is not the same as thyroxine-binding globulin (TBG)), which is cleaved by enzymes to produce active T4.

The steps in this process are as follows:

1. The Na+/I− symporter transports two sodium ions across the basement membrane of the follicular cells along with an iodide ion. This is a secondary active transporter that utilises the concentration gradient of Na^+ to move I^- against its concentration gradient.
2. I^- is moved across the apical membrane into the colloid of the follicle by pendrin.
3. Thyroperoxidase oxidizes two I^- to form I_2. Iodide is non-reactive, and only the more reactive iodine is required for the next step.
4. The thyroperoxidase iodinates the tyrosyl residues of the thyroglobulin within the colloid. The thyroglobulin was synthesised in the ER of the follicular cell and secreted into the colloid.
5. Iodinated Thyroglobulin binds megalin for endocytosis back into cell.
6. Thyroid-stimulating hormone (TSH) released from the anterior pituitary binds the TSH receptor (a Gs protein coupled receptor) on the basolateral membrane of the cell and stimulates the endocytosis of the colloid.

7. The endocytosed vesicles fuse with the lysosomes of the follicular cell. The lysosomal enzymes cleave the T4 from the iodinated thyroglobulin.
8. The thyroid hormones cross the follicular cell membrane towards the blood vessels. Monocarboxylate transporters (MCT) play major roles in the efflux of the thyroid hormones from the thyroid cells.

Thyroglobulin (Tg) is a dimeric protein produced by the follicular cells of the thyroid and used entirely within the thyroid gland. Thyroxine is produced by attaching iodine atoms to the ring structures of this protein's tyrosine residues; thyroxine (T_4) contains four iodine atoms, while triiodothyronine (T_3), otherwise identical to T4, has one less iodine atom per molecule. Each thyroglobulin molecule contains approximately 100–120 tyrosine residues, a small number of which (<20) are subject to iodination catalysed by thyroperoxidase. The same enzyme then catalyses "coupling" of one modified tyrosine with another, via a free-radical-mediated reaction, and when these iodinated bicyclic molecules are released by hydrolysis of the protein, T_3 and T_4 are the result. Therefore, each thyroglobulin protein molecule ultimately yields very small amounts of thyroid hormone (Fig 1).

More specifically, the monoatomic anionic form of iodine, iodide (I^-), is actively absorbed from the bloodstream by a process called iodide trapping. In this process, sodium is cotransported with iodide from the basolateral side of the membrane into the cell, and then concentrated in the thyroid follicles to about thirty times its concentration in the blood. Then, in the first reaction catalysed by the enzyme thyroperoxidase, tyrosine residues in the protein thyroglobulin are iodinated on their phenol rings, at one or both of the positions ortho to the phenolic hydroxyl group, yielding monoiodotyrosine (MIT) and diiodotyrosine (DIT), respectively. This introduces 1–2 atoms of the element iodine, covalently bound, per tyrosine residue. The further coupling together of two fully iodinated tyrosine residues, also catalysed by thyroperoxidase, yields the peptidic (still peptide-bound) precursor of thyroxine, and coupling one molecule of MIT and one molecule of DIT yields the comparable precursor of triiodothyronine:

peptidic MIT + peptidic DIT → peptidic triiodothyronine (eventually released as triiodothyronine, T_3)

2 peptidic DITs → peptidic thyroxine (eventually released as thyroxine, T_4) (Fig 2).

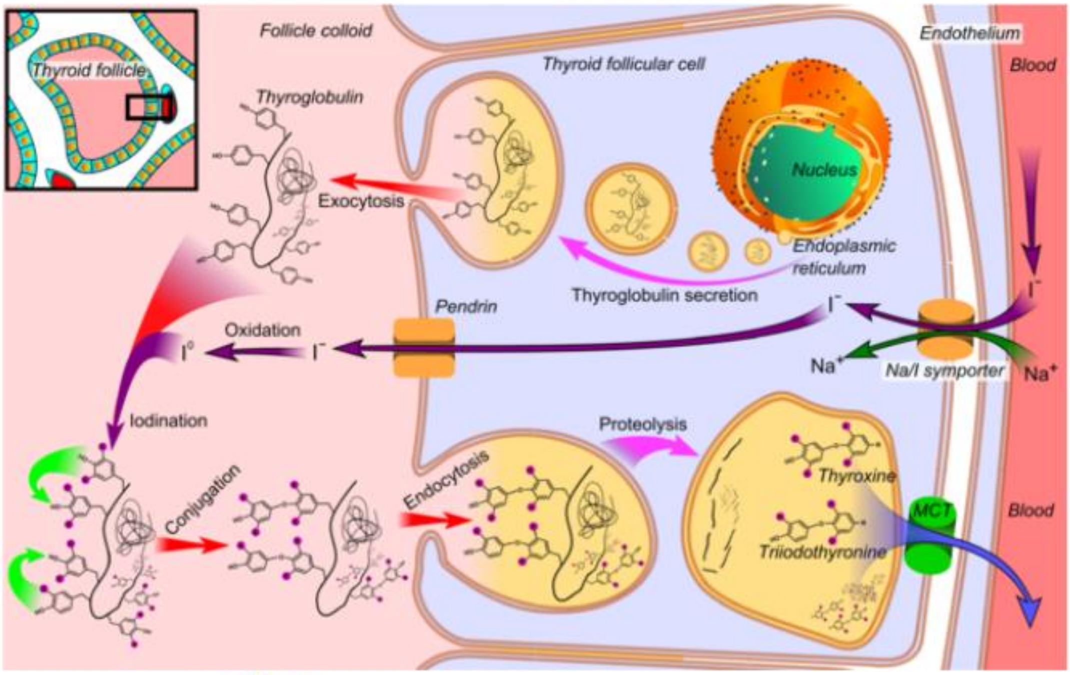


Fig 1

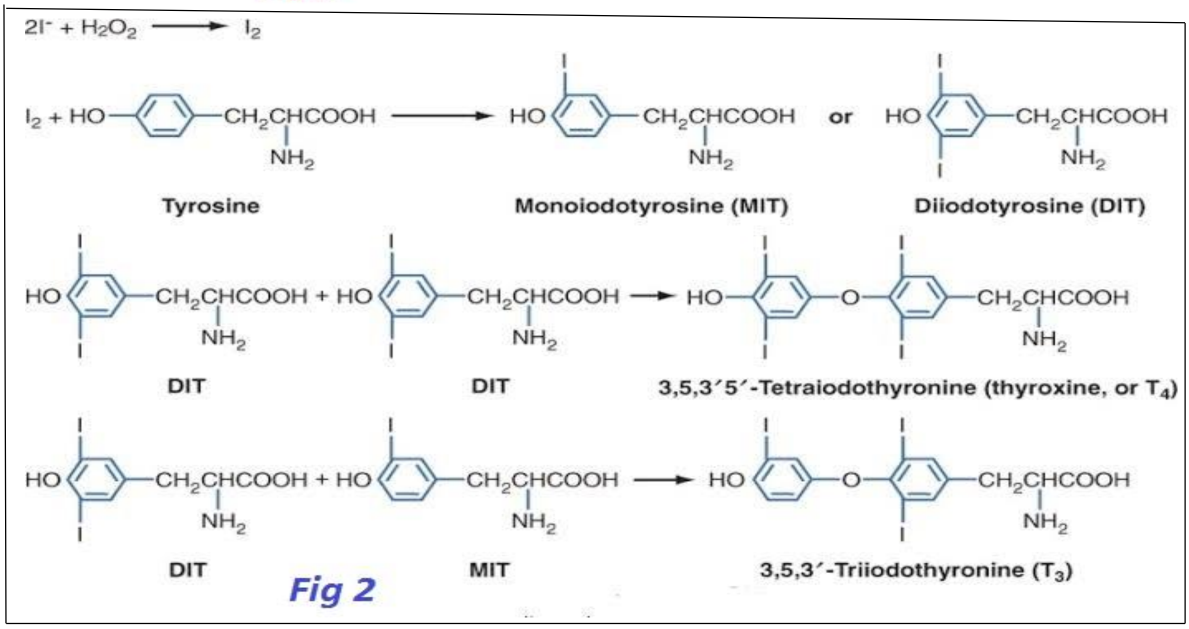


Fig 2

Functions:

The thyroid hormones act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, regulate long bone growth and neural maturation, and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness. The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism. They also stimulate vitamin metabolism.