Review Article

MINI-REVIEW: METABOLIC FUNCTIONS AND MOLECULAR STRUCTURE OF GLUTATHIONE REDUCTASE

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ABSTRACT

Glutathione reductase (GR, EC 1.8.1.7) is ubiquitous substrate specific antioxidant enzyme that reduces the disulfide (GSSG) to reduced state (GSH). Glutathione (GSH) is multifunctional tripeptide antioxidant, reduced form of oxiglutathione (GSSG); cellular GSH/GSSG ratio has been regulated by glutathione reductase. The increasing GSSG leads to cellular complications like DNA, RNA breakage and protein de-folding resulting in cell death or mitochondrial dysfunction in Liver, Kidney and cell lines. Glutathione metabolism is playing prominent role in sulfur metabolic regulation in all living cells throughout the system; the total GSH and GSSG has estimated as 300:1 ratios. GR expression suggests that increase or decrease of glutathione level causes oxidative stress in inter-intracellular surfaces. This paper reviewed that essentiality of glutathione reductase in cellular metabolic and subjected to oxidative stress protection; investigation on glutathione reductase can improve the applications in variety of fields like pre and post clinical trials, organ transplantation, blood transfusion, chemotherapy, radiotherapy and heavy drug dose. Glutathione reductase can be considered as a critical biomarker for GSH/GSSG homeostasis, cytotoxicity of mitochondria and chloroplast in addition to enhances the cell viability and stability against inter- intracellular stressors.
Keywords: Glutathione reductase (GR), GSH/GSSG, FAD, NADPH, Antioxidant.

INTRODUCTION

GSH (γ-glutamyl-cysteinyl-glycine) is three constituent of amino acids this was earlier inferred that consist of two i.e. glutamic acid and cysteine. GSH was demonstrated primary hydrogen donor since the presence of free –SH (thiol) so, easily oxidizable either anaerobically or aerobically[1]. In 1920's and 1930's various hypothesis have been proposed to highlight the reduction of oxidized peptide but however the historical development of glutathione reduction began at Warburg and Christian reduction system (i.e. crude enzyme with synthetic substrates) that confided hexose monophosphate (HMP) pathway is playing a responsible role to be involved in the reduction of GSSG[2]. Initially, reduction of GSSG was reported to TPN (triphoshopyridine nucleotide) and $DPNH_2$ (diphoshopyridine nucleotide) enzymatically by glucose 6-phosphate dehydrogenase in the presence of glucose which was later experimentally proved to be GR[3,4]. Hereafter, tremendous screening and quantification methods have been developed for both glutathione and glutathione reductase[5-9]. Therefore, the glutathione dependent mechanism has apparently been emerged due to the essential role from bacteria to plants and animals. Glutathione exists in cytoplasm, chloroplast and mitochondria in reduced form throughout the cell involved in detoxification of harmful chemical species [10]. According to modern concepts, the development of most pathology in various organs and diseases is accompanied with overproduction of reactive oxygen species (ROS) and depletion of the antioxidant system (AOS). Deficiency of GR activity has been associated with many clinical complications, including drug induced hemolytic anemia, hypoplastic anemia, thrombocytopenia, oligophrenia, homozygous hemoglobin C disease, Gaucher's disease and alpha thalassemia[11]. In the formation of GSSG is also referred to as an oxidative stress because decreasing GSH ratio affects metabolic regulatory functions resulting in lipid peroxidation, DNA breakage and protein dysfunction that causes acute inflammatory disease, cerebral malaria, rheumatoid arthritis, liver, heart and kidney failure, hemolysis and aging related diseases[12-15], immunological disorder, cancer, multiple sclerosis, AIDS, Alzheimer's, Parkinson's, osteonecrosis, atherosclerosis, pregnancy complications, male infertility and cataracts[16-19]. GR activity has apparently been found in various diseases including uremia and hepatic cirrhosis[20], cystic fibrosis[21], discrepancy in glutathione biosynthethic pathway direct to glutathionuria which revealed to pathologic effect of accumulation of GSSG lead to mental retardation[22], with advanced age may contribute to the aging process due to the increasing activity of glutathione S-transferase and glutathione peroxidase in erythrocytes and lymphocytes[9,23], hypertension[24], mitochondrial dysfunction[16], and vascular diseases[25].

Glutathione biosynthetic pathway is categorized into two parts; first synthesis of glutathione by glutathione synthase from three amino acids namely glutamate, cysteine and glycine second one, reversible redox reaction of GSSG[26]. In order to donate a reducing equivalent ($H^+ + e^-$) to other unstable molecules, such as ROS, oxidants and free radicals from the –SH group of cysteine the GSH itself become reactive (GS-) so readily reacts with another reactive glutathione to form GSSG. Hence, GSH recycles oxidized glutaredoxin to reduced enzyme; generating GSSG is retrospectively reduced to GSH[27]. In healthy cells and tissues, GSH concentration is high i. e. 3.1mg/g of tissue and is regarded as more than 90% of the total glutathione pool of but in contrast to,

GSSG exists only 10%. An increased GSSG-to-GSH ratio is considered indicative of oxidative stress[28].

BRENDA, KEGG, MetaCyc, IUBMB and BioCarta are enzyme databases which endowed with detailed physiological and chemical properties of GR the enzyme assigned under EC 1.8.1.7. The 3 D structural data can be obtained from PDB with high resolution such crystal structure available for E.coli, yeast and human. Genetic and proteomic information of the enzyme from various sources illustrated in table 1 and 2.

Table 1: Prokaryotes genomic and protein physiology

S.No	Organism	Localization	Amino acids	Transcript	Protein Size (KDa)
1.	*E. coli* [108, 109]	*	450	1.4 kb	52
2.	*Streptococcus* [110,111]	*	450	1.4 kb	52
3.	*Cyanobacteria* [49, 105]	*	458	1.4 kb	53-55
4.	*Plasmodium* [112, 103]	Schizonts	500	2.2 kb	56

*Cytoplasm

Table 2: Eukaryotes genomic and protein physiology

Organism	Localization	Amino acids	Transcript	Protein Size (KDa)
S. pombe [106]	+	465	Two exons and an intron of 55 nt, initiation site at 239 (1.4 kb)	50
Pisum sativum L. [113, 114]	*, ^	550 and 497	10 exon and 9 intron (7.2 kb)	55
Brassica [48, 60, 115]	^	502	of 17 exons and 16 introns (13-14 kbp)	54-55
A. thaliana [116, 117]	+, ^ and peroxisomes	499	1.8 kb Chromosome 3	54
N. Tabacum [118]	*, ^	557	2.2 kb	60
Oryza sativa L. [119, 60, 70]	*	496	7.4 kb;17 exons and 16 introns; 3, 2, and 10th chromosomes	53-54
Triticum durum (Wheat) [120, 121]	Endosperm *, ^	468	1.4 kb	60
Horse [122]	#	453	5.9 kb Chromosome 27	58
Rat [123, 124]	#	420	1.4 kb Chromosome 2	60
Sheep [125, 126]	Brain cells	456	1.4 kb	64
Bovine [52, 127]	# , +	519	2.3 kb Chromosome 27	55
Mouse and Human [68, 128]	*, +	500 and 522	50 kb; 12-13 exons Chromosome 8	52

*Cytoplasm, + Mitochondria , ^ Chloroplast, # Liver erythrocytes

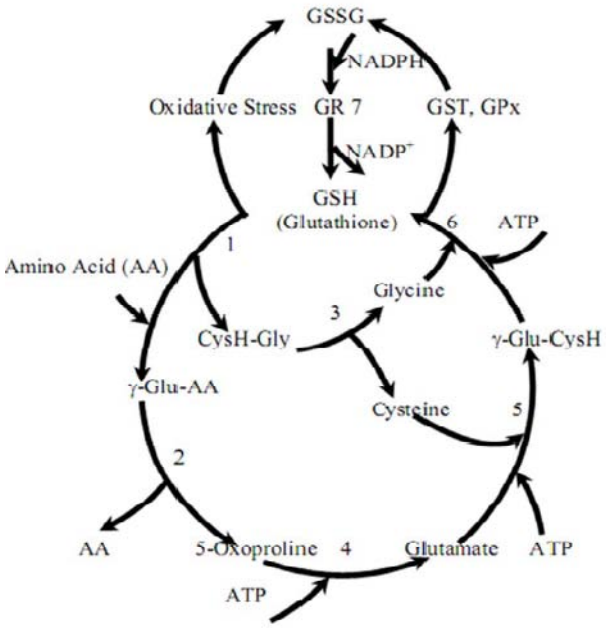


Figure 1: Metabolic and Antioxidant pathway of GSH in ϒ Glutamyl cycle; 1) ϒ -Glutamyl transpeptidase, 2) ϒ -Glutamyl cyclotransferase, 3) cysteinylglycine dipeptidase, 4) 5-Oxiprolinase, 5) ϒ-Glutamyl Cysteine synthetase, 6) Glutathione synthase and 7) glutathione reductase.

Biosynthesis and re-dox cycle of glutathione

GSH can be synthesized from γ–Glutamyl cycle in which several enzymes are involved in the synthesis and metastasis of glutathione[22,26,29-31]. Glutathione is synthesized by the consecutive action of five enzymes which directly involved in the cycle among seven rest of them recycles residue product with the addition of either amino acid or ATP to glutathione synthesis (figure 1); i) Cysteinylglycine, formed in the transpeptidation reaction which is catalyzed by γ-Glutamyl transpeptidase; ii) split into glycine and cysteine by dipeptidases (cysteinylglycine dipeptidase); iii) γ–Glutamyl Cysteine synthetase uses glutamate and cysteine link together to form a dipeptide; followed by, iv) γ-Glu-Cys is coupled with glycine by glutathione synthetase to generate GSH[32] finally, v) GSH/GSSG ratio equilibrated by GR in order to protect the homeostasis of glutathione in cytosol, mitochondria and chloroplast.

Biological functions of Glutathione

GSH is described as a mother antioxidant, a central source of cysteine amino acid first isolated from yeast, liver and muscle in 1922. GSH is major intracellular thiol compound that can involve in thiol redox state associated

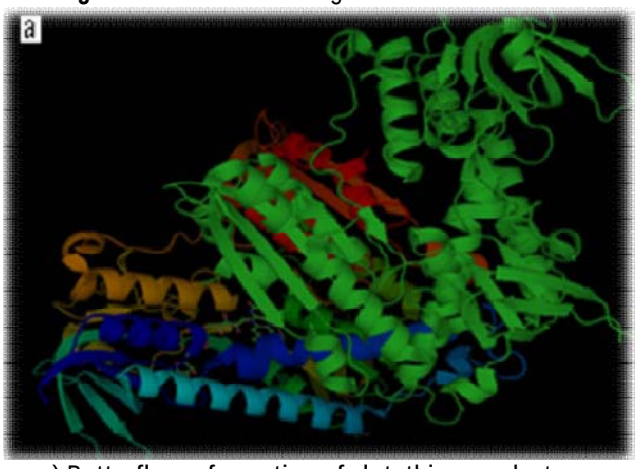
biochemical processes including protein synthesis, phosporylation, transport, stabilization of protein structures, folding, protection of cysteine residues, binding of DNA transcription factor and acceleration of H_2O_2 scavenging in redox pathway[33-37]. Most of the cellular GSH (85–90%) is present in the cytosol, with the meager amount in other organelles including mitochondria, nuclear matrix, and peroxisomes[31]. GSH is a primary substrate for glutathione peroxidase (GPx) and Glutathione S-transferase (GST) which is involved in the glutathione redox cycle[38]. GSH plays a prominent role in the formation of microtubulin spindle in cell division[39]; in the formation of the deoxyribonucleotide precursors of DNA; in maintaining the sulfydryl groups of intra cellular proteins[40]; in intracellular macromolecular assembly due to sharing thiol[41]; preserving the ability of the cell to generate ATP and to maintain membrane integrity [42]; serves as an electron donor for GPx, it catalyze toward GSH-dependent reduction of organic hydrogen peroxide (t-Butyl and LP-OOH) to water and corresponding alcohols[43]; potential factor for cytokine production**,** leukotriene and prostaglandin metabolism[31, 44, 45]; as a protective agent for enzymes involved in hydroxylation of steroids in the adrenal cortex and non-enzymatic regulator for the generation of ascorbic acid[8].

GSH is reducing equivalent for substrates of thioredoxin reductase, dehydrogenase, thiol isomerases and transferases[46-48]. GSH is required to maintain the consistent condition for nitrogen fixation and hydrogen production in cyanobacteria[49] and owning inter relationship between bacterial symbiants for example, Rhizobacteria. To perform these functions, the GSSG must be in reduced from disulfide bond between the oxidized glutathione[50, 51].

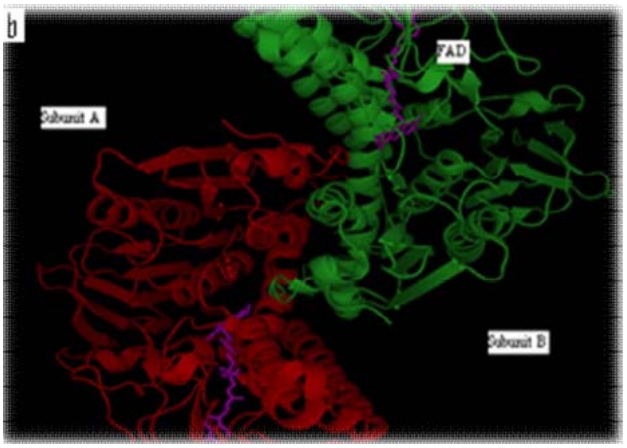
Characteristics of Glutathione reductase

GR is a potent substrate specific enzyme that belongs to a member of pyridine-nucleotide disulfide oxidoreductase, family of flavoenzymes (FAD) which plays an essential role in catalyzing the oxidized forms of glutathione (GSSG) into reduced form (GSH) mediated by NADPH source of reducing power generated from hexose monophosphate pathway[27]. The enzyme glutathione reductase has been characterized as 100-120 KDa homodimer, consists of two subunits arranged in the form of a butterfly conformation with FAD as their prosthetic group (figure 2: a and b); hence, two catalytic subunits form a single functional unit[52]. Each subunit forms four domains i.e. i) central catalytic site from N- to the C-terminal end of the polypeptide chain, ii) FAD-binding, iii) NADPH-binding, iv) central interface domains[53, 54]. The first two NADPH and FAD domains bind each other structurally and remaining site extends through C-terminal[55]. Perhaps, the electron and proton transfers happen from the *re*-side to the *si*-side of the flavin[56]. NADPH is in close contact with isoalloxazine ring of flavin referred to *re*-side of the FAD adenosine molecule stretch apart known as *si*-side. This conforma is due to transition of phenolic ring of Tyr-197 move aside from *re*-side of FAD when NADPH interact with isoalloxazine as liberated the oxidized state ($NADP^+$) Tyr become native position observed in human GR (figure 3: a, b and c).

Figure 2: 3D structure of glutathione reductase

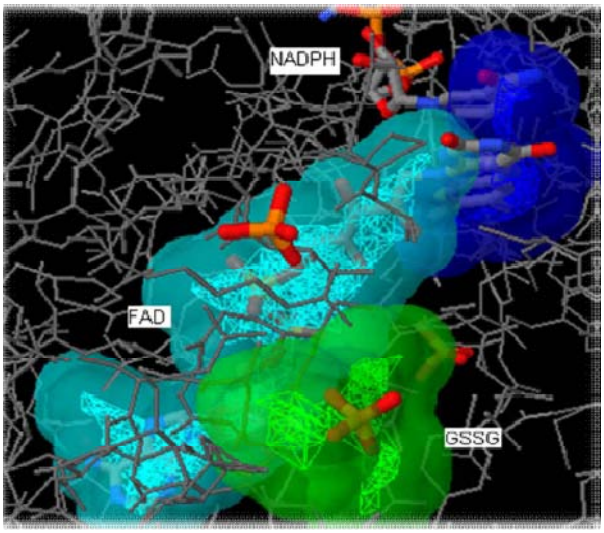


a) Butterfly conformation of glutathione reductase

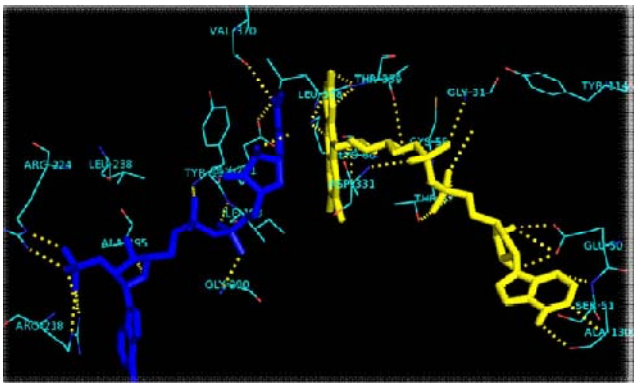


b) Subunits A (Red) and B (Green), pink highlights the FADs in both subunits.

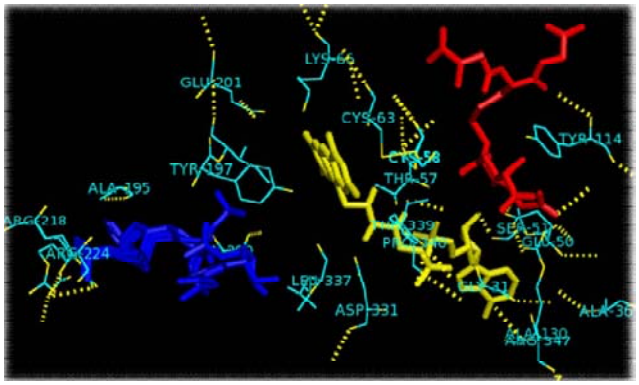
Figure 3: Geometric conformation of glutathione reductase from 3DJG and 3DK4



(a). Catalytic domains of FAD, NADPH and GSSG



(b). NADPH facing towards *re*-side of FAD (T-197 opened when reduced NADPH move toward FAD)



(c). *re*-side facing interaction broken between N7N-O4 and O7N-N3 of FAD and NADPH respectively. T-197 transist to FAD due to oxidation of $NADP^+$ elevated from domain.

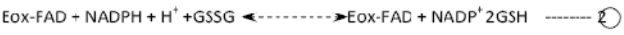
During redox active cycle, the electron transformation from NADPH to FAD is mediated through the disulfide bridge between CYs-58 and Cys-63, hence is generally known as redox active disulfide of the poly peptide. Since the absolute occupancy of ligand enthalpy of GSH-I and GSH-II of the substrate GSSG interact with redox active center[57]. The reaction ends with liberation of two reduced glutathione molecules from GSSG (Eq.1).

The localization and intermolecular interaction between electron donor (NADPH), cofactor (FAD) and substrate (GSSG) in its catalytic pocket amino acid residues (i.e. FAD-NADPH dimerization domain) are A-195, Y-197, I-198, E-201, R-218, R-224, V-270, G-290 and L-337 form polar contacts between the oxygen and hydrogen atoms in NADPH binding domain. The movement of the NADPH is regulated by Tyr-197 hence the amino acid playing vital role in the electron transfer. In FAD binding domain and GSSG binding site, G-31, Q-50, S-51, T-57, C-58, K-66, A-130, D-331, T-339 and A-37, C-58, Y-114, R-347 amino acids respectively, found with non-bonded interaction in their catalytic sites. Cysteine (58) residue commonly interacts with both FAD and GSSG in order to transfer the electron from NADPH (figure 3a, b and c).

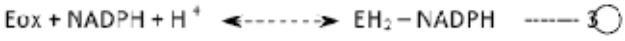
The total redox reaction occurs in three states as follow; i) GSSG binding to the catalytic domain; ii) Formation of mixed glutathione between Cys-58; iii) Release of glutathione I (GSH I) and subsequently II (GSH II). Flavin ring can be shifted in reduced form by Cys-63 in thiolate-flavin charge transfer interaction pushed (about 0.3A) towards the nicotinamide pocket[54].

Catalytic Reaction of Glutathione reductase its substrate

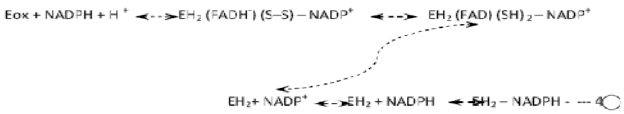
The catalytic cycle can be subdivided into two half-reactions. The ground state of the enzyme is referred to as E_{ox} where two electrons reduce the enzyme (EH_2). The first one represents a reductive reaction with the enzyme reduction by NADPH where Tyr-197 plays a role like regulatory gate for NADPH binding. Reaction No. 2 represents the whole catalytic mechanisms of GR that catalyses the glutathione disulfide into reduced glutathione mediated by FAD as cofactor and released $NADP^+$ from NADPH as electron donor. The binding positions for NADPH and GSSG are separated by the isoalloxazine ring at opposite sides of each subunit [55]. The study on the human and malarial parasitic GR has revealed with information that the reducing equivalents of NADPH are passed to GSSG via the isoalloxazine ring of FAD and the redox disulfide/dithiol center formed by two cysteinyl residues, Cys-58 and Cys-63 in the human enzyme. In this reaction, crystal and spectral data have been proved to form four intermediates namely i) oxidized enzyme (EH_2 $(FADH^-)(S\text{-}S)^{\cdot}$ $NADP^+$), ii) mixed disulfide (EH_2 (FAD) $(SH)_2$ - $NADP^+$), iii) thiolate-flavin charge transfer complex (EH_2(FAD) $(SH)_2$) and iv) (EH_2(FAD) $(SH)_2$ NADPH) [56].



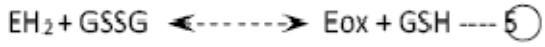
This step rises to the formation of a two-electron reduced species (*E*H2) with an oxidized FAD and a reduced dithiol center (Reaction No. 3).



First scheme of reduction reaction is charge transfer through flavin and disulfide in reductive cycle of oxidative enzyme, illustrated as Reaction 4.

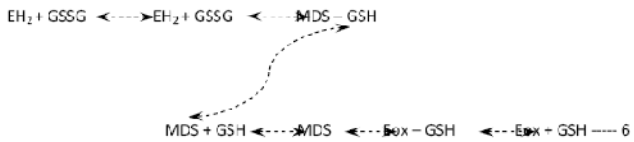


The second oxidative half-reaction is EH_2 that react with GSSG to yield two molecules of GSH to regenerate the oxidized enzyme with its active disulfide site Reaction 5.



II[nd] Scheme of oxidative half-reaction, Reaction 6 illustrates the oxidized enzyme interacts with dithiol active center to form an intermediate so-called mixed

disulfide (MDS) takes place till GSH release from the catalytic pocket. The stable intermediate is possible because of distal amino acid Cys-58 interchange thiol with GSSG.



Isoalloxazine of FAD involved in electron transfer so administration of flavin enhances the GR activity [11, 58] which depends on FAD, elevated level of flavin may affect the regulation passively[20]. The enzyme is apparently sensitive to riboflavin deficiency that drastically reduces the activity due to prolonged paucity of the flavin probably leads to inheritance effect this observed from liver and erythrocytes[59].

Biological functions and impairment consequences of Glutathione reductase in plant and animal system

High-chloroplastic/ mitochondrial expression of GR activity is showed to increase the resistance to photoinhibition and also improve tolerance against various stress such as ozone, paraquat, salt, hydrogen peroxide, chilling, Abscisic acid (*ABA),* heat treatment, heavy metals and pesticides (Methyl viologen) in cyanobacteria and *Brassica*[48, 60]. Salt stress apparently triggers the cellular glutathione level when compared to salt-tolerant transgenic plant suggests that increasing content of glutathione protects the organism from oxidative stress[61]. Moreover, over expression of GR or glutathione synthase in chloroplast is reported to increase the antioxidant capacity improve the tolerance against photoinhibitory factors in short and long life cycled plants of *N. tabacum* and wheat[62] compared to control[63].

Graminaceous and non-graminaceous plants were examined for glutathione reductase under Fe-deficient condition, where GSH scavenges ROS via the AsA–GSH cycle. Although the AsA–GSH cycle was down regulated under Fe-deficient conditions increased activity of SOD (superoxide dismutase) indicates that GR may play a role in coping with Fe-deficiency-induced oxidative stress through the GPx cycle in combination with SOD[64]. Dysfunction in GR activity leads to many cellular complications such as Chlorosis, accumulation of hydrogen peroxide and production of superoxide which decreases the GSH content in thiol homeostasis[65].

In animal kingdom, -SH oxidative stress contributes to atherogenesis, atherogenic OxLDL (oxidized low-density lipoprotein) promotes inflammatory effect by disrupting mitochondrial thiol redox state lasting in OxLDL cytotoxicity in macrophages[66, 67]. Dysfunction of mitochondria induces the evolution of free radicals which affects electron transport chain (ETC) the enzyme neutralizes the thiol leading toxicity[27, 68]. GSH deficiency leads to severe degeneration of the epithelial cells of the jejunum and intestinal colon. However, GSH have protective effect on the gastrointestinal epithelium because it served as a good source of cysteine for intracellular GSH synthesis in the gastrointestinal tract, on other apart playing a significant role in the peroxyl (-OOH) scavenging mechanism and in maintaining the functional integration of the cell membranes. It is also reported to involve in the detoxification of xenobiotics and heavy metals[69, 70].

Serum glutathione reductase has been examined in cystic fibrosis subjects (CF), obligate CF heterozygote, and control subjects that revealed to be dissimilar, mean serum GR in CF was greater than control subjects. Serum GR determined in non-CF individuals with chronic obstructive pulmonary disease (COPD) indicated different GR activity. In none of these controls or COPD was serum GR as great as the CF mean suggesting the abnormal activity of glutathione reductase to fundamentally relate to the pathogenesis of cystic fibrosis leading to immune compromised diseases like HIV/AIDS, viral infection, pneumonia and hepatitis proposed to deficiency in organization of lymphocytes, monocytes, neutrophils, macrophages induces apoptosis in $CD4^+$ T cells[30]. The fetus delivery complication subjected to Cesarean Delivery (CD) or Vaginal Delivery (VD) is said to influence the generation of free radicals where the GR activities are significant higher since low concentration of GSH[71]. Similarly, although most of the tissue possess the capacity to synthesis GSH from its amino acid precursors, the liver is major releasing source of GSH and also responsible for detoxification of endogenous and exogenous toxicants which is transported to other organs like heart, kidney and lungs syntheses less amount of GSH than liver[72]. High-fluoride concentration in water alters the blood GSH/GSSG ratio but not affect the GSH homeostasis in liver that protect from fluoride toxicity [73]. The Glutathione reductase is actively involved in cerebral glutathione homeostasis which stabilizes the interaction between neurons and astrocytes[74]. Sex steroid hormones is reported to influence glutathione redox (GSH, GR, and GPx) mechanism in dermis and epidermis leading to aging as exposed to Chemiluminescence and TBA-reactive substances that aid in generating free-radicals[75].

Low concentration of GSH in cancer patients showed a complete or partial response to chemotherapy that advanced or progressive condition in both the breast-cancer group and other tumors[76]. There is much evidence that tumor glutathione (GSH) concentration is an important factor in resistance to cancer chemotherapy and other cancer therapeutics[36, 77]. In addition to, chemotherapy and radiotherapy treated colorectal tumor patients has no significant changes because of decreasing in the level of GSH revealed to increased turnover of GSH for preventing oxidative damage in the patients [78]. Jehonathan *et al.,* (2007)[79] demonstrated that Radiotherapy and Androgen deprivation (AD) therapy stimulate stresses in radiosensitized human Prostate Cancer (PC) cells by selenite, thereby decreased antioxidant mechanism but glutathione dependent system was apparent. Surapaneni and Priya (2009)[80] have

also evidenced that the erythrocyte antioxidant enzyme, i.e. GR activity found to be increased significantly in patients with prostate cancer. Alan and Karam (2009) [81] proved that glutathione (GSH) has cytoprotective effect on the treatment of inorganic mercury (HgCl2) in neuroblastoma cells (N-2A). Transplanted malignant tumors in mice and rats were associated with increased plasma GR, latter enzyme increments appearing sooner after implantation of tumor cells and reaching levels quantitatively greater than the former. GSH can considerably influence over the severity of immediate kidney transplantation which reduces the inflammatory responses of donor kidney in the host[82].

Many report suggests that pathogenesis of several neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease, Huntington's disease, Friedreich's ataxia and amyotrophic lateral sclerosis are consequences of ROS and mitochondrial dysfunction because feeble concentration of glutathione in brain tissues [16, 83, 84]. Deficiency of GR in mitochondria leading to further loss of Electron transport chain (ETC) activity in hypertension, arthrosclerosis and liver cirrhosis[24].

Experimental determination of enzyme activity

There are two sort of quantification assays has been postulated for the GR activity. First method is UV assay this deals with the oxidation of NADPH so, the activity can spectrometrically be observed at 340 nm which can be calculated by molar extinction coefficient (E_o) 6.22 mM^{-1} cm^{-1} of NADPH[49]. Second one, is colorimetic quantification method can be measured by formation of GS-TNB complex from DTNB (5, 5' dithiobis (2-nitrobenzoic acid)) develops yellow color because of the DTNB reduction. The GR activity can be measured by visible range at 412 nm, the total activity can be calculated from E_o: 14.15 mM^{-1} cm^{-1} of TNB[85].

Although, glutathione reductase has been purified from various plant and animal sources by similar chromatographic techniques, there exists a slight variation between plant and animal with respect to ligand affinity chromatography whose lack affinity for 2', 5'-ADP sepharose in the plants since the protein sequence carries the G*X*G*XX*G fingerprint motif (Fanyi *et al.,* 1995) for example., reactive red 120 agarose for cyanobacteria. Other chromatographic purification can be done in DE-52, Polybuffer exchanger, and Sephadex G-200 or Sephacryl S-300 to isolate homogeneous GR protein through Ion-exchange, chromatofocusing and gel filtration techniques respectively[49, 52, 86].

Based on these purification steps, the purity of the enzyme yield were reported high from various sources their kinetic properties estimated with different concentration of GSSG, (0-100 μM) and NADPH (0-50 μM) the reaction velocity of the enzyme systems has given parallel lines indicating that the reaction proceeds by a branching order mechanism reported from yeast, rat liver and *Euglena* GR[34]. There are two sort of possible kinetic mechanism has been found i.e. hybrid and random BI-BI mechanism. First enzyme-NADPH and enzyme-GSSG are formed by ping pong and sequential branch order then, the product liberated from the enzyme-substrate complex[87]. After incubation with NADPH the enzyme activity was decreased about 30-50%. However, the activity could be restored to almost 100% by either GSH or GSSG and to about 60% by dithiothreitol. Preincubation with NADPH, leads to a time and concentration-dependent decrease enzyme activity[88].

Berivan and Nuray, (2008)[89] reported that pH of the enzyme in various buffer system within the pH range of 5–10 to be stable however, the optimum enzyme activity was found only in alkaline pH (7 and 8) but for plant and cyanobacterial GR activity reported at pH 9.0 in contrast to animal GR (pH<8)[90]. GR exhibits optimal activity up to 60^0C after which the enzyme activity decreases with increasing temperature[86] however, in contrast to animal GR, plant enzymes are less stable at >45^oC and had completely lost its activity at 55^oC [34]. Concentrations of $NADP^+$ - NADPH; GSH-NADPH; GSH-GSSG, and $NADP^+$-GSSG performs competitive, non-competitive and uncompetitive inhibition in different kinetic parameters though the enzyme is found in both ping-pong and sequential mechanisms of multi-substrate reaction [52] but GSH-dependent $NADP^+$ reduction is stimulated by addition of dithiothreitol.

Inhibitors of Glutathione reductase

i) N-ethylmaleimide (NEM) is used to prevent oxidation of GSH while oxidation is limits the GR activity. NEM can also be used to determine the total GSSG, referred to the potent inhibitor of GR[28].

ii) Angela *et al.,* (2007)[47] studied in parasites that GR with 10nM of Auranofin (antischistosomal compound) can rapidly inhibit the enzyme activity which resulted in the death of parasite. Other glutathione conjugate enzyme thioredoxin glutathione reductase (TGR) regarded as drug target in Schistosomiasis which is treated by praziquantel. TGR is essential for parasite survival, revealed enzymatic properties that differed from those of mammalian TGR, TrxR, and GR.

iii) TGR (isozyme) was also identified as a multifunctional oxidoreductase with remarkably wide substrate specificity[91]. The well characterized activity of enzyme TGR of malaria parasite (*P.falciparum and P. berhei*) has been postulated as new drug target (pfGR). The therapeutic concentration of methylene blue as an antimalarial agent has been demonstrated against inhibitor of pfGR[92, 93]. GSH in methylene blue treated erythrocytes therefore, elevated level of GSH tends to involve non-allosteric inhibition instead of glutamate in Ɣ-glutamylcysteine synthase binding site this can be explained by drug's effect on glutamate concentration.

iv) Study of regulation of GR activity supports by cellular concentration of citrate, 2-Oxoglutarate, and oxaloacetate but increasing intracellular above metabolites could be inhibit the enzyme activity[15].

v) *N,N*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU) has non-specific inhibitor, 2-acetylamino-3-[4-(2-acetylamino-2-carboxyethyl sulfanyl thiocarbonyl amino) phenylthiocarbamoylsulfanyl] propionic acid (2-AAPA) found >80% inhibition as a novel irreversible inhibitor that increased the neuron hydroperoxide toxicity. Some anticancer alkaloid drugs also inhibited the glutathione reductase activity[37, 94, 95].

vi) Buthionine sulfoximine (BSO), an irreversible inhibitor of ϒ-glutamylcysteine synthetase**,** has been widely used to inhibit GSH synthesis. BSO is an advanced anolog of methionine sulfoximine (MSO), first known as an inhibitor of glutamine synthetase and later of ϒ-glutamylcysteine synthetase[17, 29].

vii) Cycloheximide, Actinomycin D and cordycepin decline the enzyme-substrate interaction so that elevate the GSSG level which consequently affects the protein synthesis[33]. Thiol inhibitors and metal ions such as Hg2+, Z^{n2+} and C^{u2+} markedly inhibited the enzyme activity[34]; the polyamines decreased the GR activity to different degrees, depending on time after application, type of compound and their concentration[96].

viii) 5-aminolevulinic acid (ALA) is an essential precursor in the biosynthesis of porphyrins such as chlorophyll and heme, low concentrations of ALA could enhance the antioxidant level in spinach, potato, pakchoi and ginkgo improve plant's tolerance to cold and salinity stresses. ALA reduces GSSG/GSH ratio due to inhibition of GR activity when grow in saline condition[97, 98].

Scope of the study of glutathione reductase

GR is exclaimed as antioxidant defense system since renowned intrinsic functions in cellular metabolic pathways. Giving GSH as health supplementary hoped that reduce the side effects of radiotherapy and chemotherapy treatments which enhances the cell viability[36]. During the transplantation of organs that believed to raise the stress resulting in evolution of radicals because of the immunological responses. Thereby, enhancing GSH synthesis and GR activity certainly replaces the thiol homeostasis in the cell that increased viability of immune cells, assembly of macrophages and neutrophils by the way prevent the oxidative damages and inflammatory effects [30]. Cellular GSH deficit induces the apoptosis-signaling mechanism in cancerous cell lines evidenced with treated hematopoietic malignant cells by nordihydroguaiaretic acid (NDGA). Impairment of homeostasis of GSH:GSSG and TRS can be considered as cytotoxic effect in therapeutic treatments with anticancer drugs [99]. Inversely, GR and thiol dependent antioxidant defense mechanisms seize many anticancer alkaloid drugs effect on glutathione oxidation and mitochondrial depolarization but not free radical or ROS generation is appreciable for cancer treatments [94, 100]. GR activity can be increases the drug metabolism and sensitivity examined in GR-activity deficient RBCs against antimalarial drug because of that most drug failure[73, 101]. Hence, it is identified as new drug target for malaria and schistosomiasis which leads to novel drug discovery[92, 102, 103].

Optimum level of cellular GSH content can substantially be reduced the severity of pathologic effect of stress. GR is a key antioxidant in plant and microbes involved in detoxification of xenobiotics and pollutants over the paddy and wheat cultivation area. Increasing the GR activity could acclimatize with drought, salt, light, temperature and other unfathomable conditions like lipid peroxidation, solute leakages, and the RNA and protein synthesis[33]; induces photosynthesis, glutathione-ascorbate cycle, heterocyst differentiation and N2 fixation dependent of glutathione since they are playing a integrative role in metabolic activities[49, 104, 105]. Transgenic plant variety and overexpression of transgene causes cytotoxicity the enzyme hinder these lethal effects that evidenced from graminaceous and non-graminaceous plants. These plant varieties are highly light sensitive to overwhelm of oxidative damages due to the over production of ROS and free radicals which can be conquered by expression of glutathione redox system. Chloroplastic GR over expressions withstand even in photoinhibitory conditions reported in many plant and microbes like yeast, Brassica, Tobacco and barley[64, 106, 107].

CONCLUSION

Every living organisms has its own protective mechanism against the consequences of harmful agents such as pro-oxidants, oxidants, reactive oxygen species and free radicals because of stress influences of extra and intra or inter molecular factors. Antioxidant defense system is unique, discrete mechanism defend the organism from toxic compounds. This ubiquitous defense mechanism can be sub-divided into two, enzymatic and non-enzymatic system. Both are involved in detoxification of ROS and free radicals either directly or indirectly. These mechanistic compound or molecules belongs to non-enzymatic category since it acts as electron acceptor or donor but enzyme like SOD, catalase, peroxidases and transferases involves in detoxification by linear pathway networks so-called enzymatic mechanism. GSH is a notorious antioxidant in glutathione metabolism, involved in most cellular and molecular function. Moreover, GSH-dependent enzymes utilizes GSH as substrate for their functions, therefore donating electron from reduced GSH itself become reactive these two reactive species (free radicals) forms glutathione disulfide (GSSG). Accumulating GSSG is referred to as oxidative stress and cytotoxic hence it is maintained at low strength compared to GSH,

homeostasis of glutathione pool is regulated by GR. Utilization of hydrogen from reduced GSH is driven to forming GSSG simultaneously decreases the reduced GSH the disparity of homeostasis results in adverse effect probably cell death. The normal glutathione metabolic pathway is completely dependent of GSH level so, glutathione pool had has to be replenished the optimum level of reduced GSH. GR is a specific enzyme that catalyses the GSSG driven back to reduced GSH form in total glutathione content therefore, GR so-called factory of GSSG reduction.

Perhaps, modulation in the enzyme activity suggests in response to oxidative stress, estimation of GR activity in various diseases revealed to clinical bio-marker. The therapeutic treatment such as carmustine to brain tumor treatment, chemotherapy, radiotherapy, Androgen deprivation (AD) therapy, diseases or disorders and cancers (prostate, Breast, colon, brain) has linked to GSH, GSSG and GR activity because the above treatments of the particular diseases are revealed to high energy constituents leads to side effects like toxicity. GSH could be involved in drug mentabolism during chemotherapic treatments in order to form thiol-conjugates for excretion of unmetabolized residual molecules to reduce the toxicity of the drug. Oxidative stress can also be raised while transplantation, blood transfusion, and clinical trials because these external constituents cause negative effect to the regular system. Although these treatments harm to the normal cell metabolic activities, playing defense role in glutathione metabolism support to withstand by resistance.

GR has been well characterized from various sources that proved to functions, structure and kinetic parameters were consistent because the evolutionary close relationship from bacteria to mammals. Although having similar structure and function properties, the primary structure of the protein shares least homology which could be considered to identify a novel drug candidate against pathogenic organisms. This critical information about GR in the glutathione dependent metabolism has been disclosed an opportunity to screen novel inhibitor based on bioinformatics approach because identified certain inhibitors interact non-specific sites, such irreversible interaction leads to neurotoxicity. The enzyme had been studied in limited sources particularly in pathogenic organisms so existing information in the databases is insufficient. The more experimental and computational methods are required to analyze enzyme-substrate interaction, GSSG coupling, transition of T-197 and thermodynamic changes of the enzyme.

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