8-2018

Glutathione: A small molecule with big sense

Cristina E. Raya
The University of Texas Rio Grande Valley

Debasish Bandyopadhyay
The University of Texas Rio Grande Valley, debasish.bandyopadhyay@utrgv.edu

Recommended Citation

Debasish Bandyopadhyay et al. (2018), Glutathione: A small molecule with big sense. Int J Pharm Sci & Scient Res. 4:5, 39-44.

BioCore Exploring Scientific Community

International Journal of Pharma Sciences and Scientific Research

Review Article ISSN 2471-6782

Glutathione: A small molecule with big sense

Cristina E. Raya, Debasish Bandyopadhyay*

Department of Chemistry, The University of Texas-Rio Grande Valley, 1201 West University Drive, Edinburg, Texas – 78539, USA

Abstract

Glutathione, a peptide found in microbes, plants and animals including human plays a key role in maintaining healthy cells. The peptide exists in both reduced and oxidized forms. Synthesis of GSH occurs in the cytosol of cells, and the extent of glutathione synthesis relies on various factors, such as amino acid availability, protein activity etc. Once synthesized, glutathione exists in both forms: oxidized (GSSG) and reduced (GSH). Oxidized glutathione characterized by its disulfide linkage. On the other hand, presence of a thiol group characterizes the reduced glutathione. This thiol makes the tripeptide an essential component of health; it makes glutathione a free radical scavenger, or an antioxidant. Disturbance in glutathione synthesis or imbalance in GSH to GSSG ratio results in physiological conditions due to build up oxidants in cells. Imbalance might take place because of physiological conditions, or may result from hereditary conditions, or a disease developed from lifestyle. Dietary glutathione supplements are now being marketed. A concise overview of various aspects of glutathione in human body is presented in this mini-review.

Keywords: Glutathione, Amino acids, Antioxidant

Corresponding author: **Debasish Bandyopadhyay**

Department of Chemistry, The University of Texas-Rio Grande Valley, 1201 West University Drive, Edinburg, Texas – 78539, USA

Tel: +1(956)5789414,

Email: debasish.bandyopadhyay@utrgv.edu

Citation: Debasish Bandyopadhyay et al. (2018), Glutathione: A small molecule with big sense. Int J Pharm Sci & Scient Res. 4:5, 39-44.

Received: July 21, 2018

Accepted: August 01, 2018

Published: August 08, 2018

Introduction

Glutathione is a peptide composed of three nonessential (not essential to the human diet as body can synthesize these amino acids), amino acids viz. glutamic acid, cysteine, and glycine. In body glutathione exists in both oxidized and reduced states. The reduced form of glutathione is a tripeptide known as L-gamma-glutamyl-L-cysteinyl-glycine GSH, or glutathione, and the oxidized state is another peptide known as glutathione disulfide, oxidized glutathione, or GSSG. [1-3]. Reduced GSH is comprised of three amino acids (glutamic acid, cysteine, and glycine) linked by two peptide bonds: one between glutamic acid and cysteine and the other between cysteine and glycine (Fig. 1). Glutathione's peptide bonds leave the amino and carboxylic acid groups of the glutamic acid, cysteine's thiol group, and glycine's carboxylic acid exposed making the tripeptide hydrophilic nature [4]. The tripeptide's thiol group is important in biological and biochemical aspects because it is easily deprotonated, which makes it very reactive [5]. GSSG does not have this thiol group; instead, it has a more stable disulfide bond that links two GSH.

Presence of glutathione is more abundant in the eukaryotic domain than in the prokaryotic domain. The presence of GSH in prokaryotes is limited to cyanobacteria, proteobacteria, and a few strains of gram-positive bacteria [6]. All mammals, including humans, utilize GSH and GSSG intracellularly and extracellularly throughout their lifetime to carry out various functions essential to maintain the organism's health intact [7]. Because of their various functions, GSH can be labeled as an antioxidant, cysteine reservoir, and cell modulator of processes like DNA synthesis, free radical scavenger, and regulator of thiols in proteins [7].

Discussion

Glutathione Synthesis

Humans, as do all GSH containing organisms, must synthesize glutathione. Synthesis of glutathione occurs in the cytosol of cells. This synthesis happens in two steps, which requires its three integral amino acids, ATP, and two enzymes: glutamate synthase ligase (GCL) and glutathione synthase (GS) [7, 8]. The first step in glutathione biosynthesis requires catalytic enzyme GCL, an enzyme whose genetic codes differ amongst organisms the human GCL has two subunits and the gene is GCLC (Glutamate—cysteine ligase catalytic subunit) [9]. Essentially, in the first reaction GCL catalyzes glutamate and cysteine to give glutamyl-cysteine [10]. Aside from the two amino acids, this reaction has two more reactants that allow the reaction to move forward, ATP and a metal ion (either Mn^{2+} or Mg^{2+}) [7]. As the presence of Mn^{2+} or Mg^{2+} is essential in the reaction, this first catalytic reaction is said to be the limiting step in GSH biosynthesis. Glutamyl-cysteine, glycine and ATP are all reactants, which undergo the enzymatic catalytic reaction of glutathione synthase (GS) which produce GSH and ADP+ Pi [7].

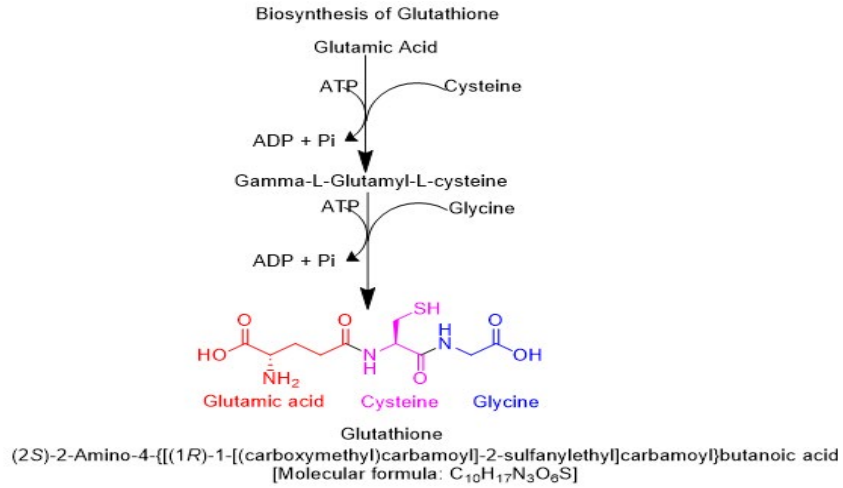


Figure 1: Structure and biosynthesis of glutathione

Factors that influence Glutathione synthesis

The synthesis of glutathione is influenced by a few factors, which include, but are not limited to amino acid availability and enzymatic activity.

Amino Acid Availability

Biosynthesis of glutathione is impossible without all three of its amino acids in the cytosol, so the availability of its precursors and the amino acids themselves is crucial. Amino acids exist extracellularly and require amino acid transport systems to get through the cellular membrane and into the cytosol where they take amino acid form. The first step in glutathione biosynthesis requires glutamate, so the availability of its precursor glutamine and the amino acid (in the cytosol) itself are of utter importance in this process. System N (SNAT3), SNAT1 (System A subtype), Alanine, serine, cysteine-preferring transporter 2 (ASCT2), and membrane transporter B0 AT1 (SLC6A19) are all systems which enable the transport of glutamine across the cellular membrane [11]. Transport systems are also a key factor in the availability of amino acids and their precursors, and are therefore a key factor in glutathione synthesis as well.

Cysteine

The most important aspect of GSH is its thiol group; in fact, it is the main source of non-protein thiols for the majority of aerobic organisms. The thiol is derived from its cysteine amino acid, and making the availability of cysteine critical in GSH synthesis, and influences the rate of GSH synthesis [12]. Availability of this amino acid is determined by many factors like conversion of homocysteine to cysteine, the individual's diet, protein metabolism, activity of the liver, the transsulfuration pathway, membrane transport and so on [7].

Glycine

Glycine is the last amino acid to be required in glutathione biosynthesis, making it a rate-limiting factor[12]. Gamma-glutamyl and glycine levels are inversely proportional, so when glycine levels are too low there is an accumulation of gamma-glutamyl in tissues [12]. Therefore preventing the completion of glutathione biosynthesis. Glycine precursors include serine, threonine, and dietary glycine making them an important part of glutathione synthesis as well [13].

Enzymatic activity

Glutathione biosynthesis requires the catalytic activity of enzymes glutamate cysteine ligase (GCL) and glutathione synthase (GS) and in some cases, gamma-glutamyltranspeptidase (GGT) this enzyme is unique to the external surface of epithelial cells and disruption of their activity results in disruption of glutathione synthesis [7].

Glutamate-cysteine ligase (GCL)

GCL is responsible for carrying out the limiting reaction in glutathione synthesis. In some organisms (including human), this catalytic enzyme is composed of a heavy and a light subunit known as GCLC and GCLM respectively, and is responsible for synthesizing gamma-glutamyl-cysteine to make it a rate-determining factor for GSH synthesis. In its synthesis, GSH determines the activity of GCL by taking on the role of a feedback inhibitor [14, 15]. Each subunit carries out its own function GCLC is responsible for catalytic activity and GCLM is the GCL's modifier subunit. Activity of both subunits' transcription is controlled by cellular stimuli [16]. Krejsa et al. reported that GCLC rapidly becomes activated when there is glutathione depletion (oxidative stress) in the cell [16]. It was also been reported that an increment in GCLC gene transcription and GCLC mRNA levels are linked to oxidative stress in the cell [7]. Oxidative stress, with a few exceptions, causes damage to cells, and essentially leads to pathophysiological conditions in the body. GCLM and GCLC subunits are not present in equal amounts in tissue; instead, there is a GCLC/GCLM ratio. The ratio varies amongst organs. It was reported that the ratio is as low as 1.5 in the spleen and goes as high as 7.0 in the brain [17]. Regardless of the variability of ratio amongst various tissues, they all have a higher amount of GCLC and, with the exception of the kidneys, GCLM is limiting in all tissues. GCLC compensates for absence of GCLM, but the glutathione level in the corresponding tissue does not necessarily embody the absence or presence of GCLM. Glutathione levels in the tissue are a combination of the amount of glutathione effluxed and accumulated [17]. In addition, GCL activity is affected by Mn^{2+} or Mg^{2+} ions, because these ions form complex with ATP. ATP-magnesium or ATP-manganese complexes serve to increase the binding energy between it and the enzyme. GCL is able to form gamma-glutamyl-cysteine from glutamine and cysteine with help from ATP-Mn or ATP-Mg complex. Glutamine contains an ATP-independent Mg^{2+} coordination site, which allows it to bind to GCL using the ATP-Mg complex regardless of its abnormal pocket for the alpha-carboxylate [18]. Without these cofactors, the complex will not form and GSH synthesis will not occur because the glutamine will be unable to bind to GCL.

Glutathione synthase (GS)

Glutathione synthase (GS) catalyzes the last step in GSH formation. Activity of the enzyme varies throughout the body, and is greater than that of GCL in most tissues, but can be limiting in tissues other than the liver [7]. The enzyme is one of the few modulators that control glutathione levels. GS activity corresponds to GCL's capacity to carry out GSH synthesis [7]. Unlike GCL, GS is not directly inhibited by GSH.

Gamma-glutamyltranspeptidase (GGT)

Gamma-glutamyltranspeptidase participates in the gamma glutamyl cycle as a surface membrane protein. GGT is significant in GSH synthesis because it provides the reaction with a rate limiting substrate, cysteine. The enzyme is able to degrade extracellular GSH and have a resulting cysteine. The enzyme is therefore able to regulate the amount of GSH that gets synthesized.

OX and REDOX reactions between GSH (reduced form) and GSSG (oxidized form)

GSH does not come to exist solely through GSH synthesis. Various reactions account for the presence of GSSG (Fig. 2) and GSH in cells. Oxidation/reduction reactions play a key role in the availability and interconversion of reduced GSH and oxidized GSSG. The structure of reduced GSH varies from that of GSSG in that it has a cysteine, which contains a thiol group instead of a cystine molecule, which contains a disulfide bond.

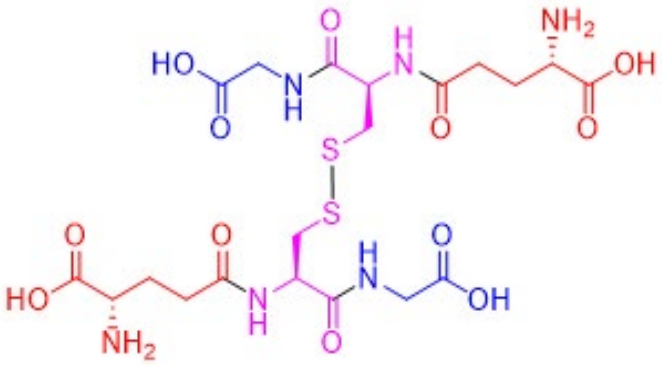


Fig. 2: Structure of Glutathione disulfide (GSSG)

Red-ox reactions, through enzymatic catalysis, occur on the thiol group of GSH and disulfide bond of GSSG interconverting these two. In the oxidation of GSH (two GSH are required) to produce GSSG, the thiol groups lose their hydrogens and by doing so the thiol groups loose electrons. Loss of electrons in a molecule means that it gets oxidized. The two cysteines become a cystine [19]. To get back to its reduced form, GSSG accepts electrons by the addition of hydrogen to sulfur. Oxidation and reduction of glutathione peptides are observed in various biological pathways. The pentose phosphate pathway exemplifies such reaction. In this pathway, GSSG is reduced to two GSH molecules by the enzymatic activity of glutathione reductase and addition of hydrogen's electron from the oxidation of NADPH to NADP+ [20, 21]. This reduction reaction is utilized during cell detoxification, a process that aims to rid of harmful free radicals. In detoxification of the cells, GSSG and GSH are interconverted by the enzymes glutathione peroxidase and glutathione reductase in a cyclic manner. Two GSH molecules from the pentose phosphate pathway become oxidized by binding to hydrogen peroxide and getting catalyzed by glutathione peroxidase. Thereafter, GSSG gets reduced in a similar fashion as in the pentose phosphate pathway, and the cycle repeats [20]. GSSG actively gets effluxed from the cell, unlike GSH that exists intracellularly to participate in detoxification; it is crucial to maintain a regulation of the abundance of GSH in the cell and GSSG that is effluxed from the cell [22].

Reactive oxygen species (ROS) homeostasis

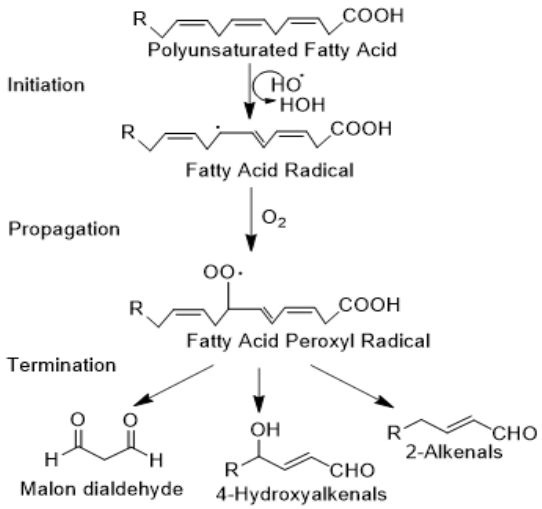
Reactive oxygen species, which include free radicals, are a group of molecules with signaling and damaging abilities. [23, 24] Homeostasis of ROS is essential in the well-being of a cell and therefore an organism. Oxidative stress occurs when the free radical production exceeds the counteracting ability of antioxidants. Free radicals are highly reactive molecules with at least one unpaired electron, which create an imbalance in cell component molecules. In biological functions, these are oxygenated molecules with unpaired electrons at the outer shell. Production of such molecules can result from partial oxygenation, as is seen in oxidative phosphorylation. These highly reactive species that result from oxidative phosphorylation are superoxide anions and hydroxyl radicals production of radicals are not limited to the oxidative phosphorylation reaction. An increment of ROS signals the cells for a redox reaction. Thiol containing molecules enable relieve in this stress by binding to these radicals and eliminating them from the body; they are said to detoxify and are known as antioxidants. These groups can be derived from cysteine residues, making the amino acid significant in the detoxification process. GSH's thiol, derived from its cysteine amino acid, makes the tripeptide an important, non-protein antioxidant that acts in a cell to try to get ROS levels back to homeostatic levels [23]. If ROS homeostasis level is not maintained appropriately, that is if ROS production exceeds the ability of antioxidants too much, cells begin to undergo oxidative stress. ROS can lead to cell damage, because of their high reactivity. These highly reactive species readily oxidize biomolecules like DNA, lipids and protein [25].

Effects of ROS on Protein

Reactive oxygen species are able to disrupt a protein's physical and chemical properties by covalently modifying (oxidizing) them. Proteins are large chains of covalently bonded amino acids [26]. Susceptibility to oxidation increases at sulfur containing side chains of cysteine and methionine, but all other amino acids are also able to undergo ROS oxidation. Physical evidence of this disruption in protein structure is evident; oxidized proteins become fragmented or form a protein-protein cross linkage [26].

Effects of ROS on Lipids

Oxidization of lipids occurs in lipid containing cell structures like the lipid bilayer, and lipoproteins [25]. These biomolecules are able to undergo oxidation through a process called lipid peroxidation. Free radical mediated oxidation, free-radical independent non-enzymatic oxidation, and enzymatic oxidation are the three mechanisms that enable lipid peroxidation to occur [27]. Overall, the reaction occurs in three steps (Scheme 1). In the first step, formation of a carbon-centered lipid radical is attained by removal of an allylic hydrogen, which then undergoes oxidization to form a peroxy radical. Subsequently, it (peroxy radical) reacts with another lipid by removing its hydrogen – the peroxy radical becomes a hyperoxide and the lipid it reacted with becomes a carbon-centered lipid radical. A chain reaction occurs amongst the lipids in these first two steps known as initiation and propagation respectively. The third step, namely termination, does not occur without mediation of an antioxidant [28].



Scheme 1 Schematic diagram of lipid peroxidation

Effect of ROS on DNA

DNA is not an exception to the oxidized by ROS. Oxidation occurs on DNA bases viz. cytosine, adenine, guanine and thymine; resulting in DNA damage. Addition of the oxygen has the ability to change the structure of the DNA bases. Oxidation by ROS is more common in guanine and cytosine bases: guanine oxidizes to produce 8-hydroxyguanine or 2, 6-diamino-4-hydroxy-5-formamidopyrimidine. Cytosine undergoes oxidation to produce 5-hydroxyhydantoin. Deoxyribose is also prone to oxidation.

Physiological conditions from the deficiency of GSH

GSH acts as an antioxidant in the body by binding to ROS, which have the ability to oxidize biological macromolecules and therefore causes cellular damage and subsequent cell death. Excretion of ROS from cells is essential to improve cellular and tissue health. Physiological conditions and aging have been linked to surplus of reactive oxygen species in the cytosol, or oxidative stress. Oxidative stress, as mentioned previously, occurs when the capacity of an antioxidant is unable to keep up with the production of ROS. Glutathione is an important, thiol containing, non-protein antioxidant for cells [23]. The tripeptide is able to alleviate the high concentration ROS in cells which in turn helps diminish the oxidation of macromolecules. Oxidation of macromolecules leads to cell damage and death, and if it is significant enough physiological change takes place in several ways. Oxidized DNA, may directly create harmful biological responses because of the newly formed mutagenic as well as toxic products [29]. Lipid oxidation by ROS is also harmful that ultimately leads to cell health. A study conducted by Wong-Ekkabut et al. supports the idea that oxidized lipids are able to increase membrane permeability, which is one of the major underlying causes of cell membrane damage [27]. Mechanisms of various neurodegenerative disorders, including the lipid peroxidation, were studied by Shichiri et al. This study identified GSH as an underlying factor of such pathological conditions because it is able to regulate the extent of ROS that reduces oxidative stress, so its availability is crucial to an individual's health [30]. It was indicated by a study that an increment of GSH in brain might help to get rid of neurodegenerative diseases [31].

Supplements

Because of its importance in health, oral supplements of GSH have been developed [32]. Various studies have been conducting by the scientists worldwide over the years to determine the efficiency of such supplements. In a study conducted by Allen and co-workers showed no significant difference in the level of GSH was observed on administering oral supplement in healthy adult human [33]. Another approach was made by increasing the level of this antioxidant. This study analyzed the effects of taking supplemental GSH to increase the availability of GSH. As described earlier, glycine is used in the last step of GSH synthesis [12]. It was found that GSH level was increased in animals who took supplemental glycine [12]. It was predicted that supplemental glycine might be able to prevent complications like diabetes, cardiac hypertrophy, aiding the control of metabolic syndrome amongst other physiological conditions [12]. Clinical studies of these supplements must be made to see how supplements affect individuals with physiological conditions [12].

Conclusion

Availability of GSH is determined by various factors, including its synthesis and ox/redox conversion from GSSG. GSH synthesis is also

dependent on several aspects including catalytic proteins, amino acid availability, and interconversion between GSH and GSSG. The enzyme GS takes part in the second catalytic activity of GSH synthesis, so without its production of the tripeptide is prevented. GS deficiency can lead to hemolytic anemia, ataxia, amongst a few other conditions. GSH is also a key factor in neurological disorders. In other words, this tripeptide protects the brain, and disorder occurs on falling GSH level. Oxidative phosphorylation enhances ROS production in healthy cells, that causes ionization radiation, inflammation, and metals from fenton reactions and other chemicals including drugs are synthesized in the liver. Deficiency of GSH leads to an increment in ROS, which in turn affects healthy cells and subsequent disease in individuals.

Conflicting interests

The authors have declared that no conflict of interests exist.

Acknowledgement

Thanks are accorded to the Department of Chemistry (College of Sciences), The University of Texas Rio Grande Valley.

References

1. Lushchak, V. I. Review Article Glutathione Homeostasis and Functions: Potential Targets for Medical Interventions. Journal of Amino Acids [Online] 2012, 2012, Article ID 736837. https://www.hindawi.com/journals/jaa/2012/736837/ (accessed July 1, 2018).

2. Pubchem Open Chemistry Database. https://pubchem.ncbi.nlm.nih.gov/compound/glutathione#section=Top (accessed April 27, 2018), Compound Summary for CID 65359, Glutathione.

3. Pubchem Open Chemistry Database. https://pubchem.ncbi.nlm.nih.gov/compound/oxiglutatione#section=Top (accessed July 20, 2018), Compound Summary for CID 65359, Oxiglutatione.

4. Schaur J. R.; Wonisch W. Chemistry of Glutathione. In Significance of Glutathione to Plant Adaptation to the Environment; Grill, D., Tausz, Michael M., de Kok, L.J., Eds.; Plant Ecophysiology; Springer Netherlands: Dordrecht, Netherlands; Vol. 2, pp. 13-26.

5. Poole, L. B. The basics of thiols and cysteines in redox biology and chemistry. Free Radic Biol Med. 2015, 80, pp. 148-157.

6. Zechmann B.; Lucija, A.T.; Fulgosi, H. H. Subcellular distribution of glutathione and cysteine in cyanobacteria. Protoplasma [Online] 2010, 246, pp. 65–72. https://link.springer.com/content/pdf/10.1007%2Fs00709-010-0126-8.pdf (accessed July 14, 2018).

7. Lu, S. C. Regulation of Glutathione Synthesis. Molecular Aspects of Medicine 2009, 30, pp. 42-59.

8. Lu, S. C. Glutathione Synthesis. Biochim Biophys Acta. 2013, 1830, pp. 3143-3153.

9. UniProt. https://www.uniprot.org/uniprot/P48506 (accessed July 16, 2018), UniProtKB - P48506 (GSH1_HUMAN).

10. Yang, Y.; Dieter, M. Z.; Chen, Y.; Shertzer, H. G.; Nerbert, D.W.; Datlon, T. P. Initial Characterization of the Glutamate-Cysteine Ligase Modifier Subunit Gclm(−/−) Knockout Mouse: NOVEL MODEL SYSTEM FOR A SEVERELY COMPROMISED OXIDATIVE STRESS RESPONSE. The Journal of Biological Chemistry [Online] 2002, 277, pp. 49446-49452. http://www.jbc.org/content/277/51/49446.full.pdf (accessed July 3, 2018).

11. Bungard, B. I.; McGivan J.D. The transport of glutamine into mammalian cells. Frontiers in Bioscience [Online] 2007. https://www.bioscience.org/2007/v12/af/2109/fulltext.htm (accessed June 30, 2018).

12. DiNicolantonio, J. J; McCarthy, M.F.; O'Keefe, J.H. Dietary Glycine Is Rate-Limiting for Glutathione Synthesis and May Have Broad Potential for Health Protection. PubMed [Online] 2018, 18. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5855430/ (accessed July 15, 2018).

13. Chao, F.; Delwiche C.C; Greenberg, D.M. Biological precursors of glycine. Biochimica et Biophysica Acta 1953, 10, pp. 103-109.

14. Biterova, E. I.; Barycki, J. J. Structural Basis for Feedback and Pharmacological Inhibition of Saccharomyces cerevisiae Glutamate Cysteine Ligase. Journal of Biological Chemistry [Online] 2010, 285, pp. 14459. http://www.jbc.org/content/285/19/14459.full.pdf (accessed July 7, 2018).

15. Meister, A. Selective modification of glutathione metabolism. Science [Online] 1983, Vol. 220, Issue 4596, pp. 472-477. http://science.sciencemag.org/content/220/4596/472/tab-pdf (accessed April 23, 2018).

16. Krejsa, C.M.; Franklin, C.C.; White, C.C.; Ledbetter, J.A.; Schleven, G.L.; Kavanagh, T.J. Rapid Activation of Glutamate Cysteine Ligase following Oxidative Stress. Journal of Biological Chemistry [Online] 2010, 285, pp. 16116–16124. http://www.jbc.org/content/285/21/16116.full.pdf (accessed July 5, 2018).

17. Chen, Y.; Shertzer, H.G.; Schneider, S.N.; Nebert, D.W.; Dalton, T.P. Glutamate Cysteine Ligase Catalysis DEPENDENCE ON ATP AND MODIFIER SUBUNIT FOR REGULATION OF TISSUE GLUTATHIONE LEVELS Journal of Biological Chemistry [Online] 2005, 280, pp. 33766–33774. http://www.jbc.org/content/280/40/33766.full.pdf (accessed July 1, 2018).

18. Biterova, E. I.; Barycki, J. J. Mechanistic details of glutathione biosynthesis revealed by crystal structures of Saccharomyces cerevisiae glutamate cysteine ligase. Journal of Biological Chemistry [Online] 2009, 284, pp. 32700–32708. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2781686/pdf/zbc32700.pdf (accessed July 24, 2018).

19. Nelson, L. D.; Cox, M. M. Lehninger Principles of Biochemistry 7th Edition; NY : W.H. Freeman and Company ; Houndmills, Basingstoke : Macmillan Higher Education: New York, New York, 2017; pp. 80.

20. Nelson, L. D.; Cox, M. M. Lehninger Principles of Biochemistry 7th Edition; NY : W.H. Freeman and Company ; Houndmills, Basingstoke : Macmillan Higher Education: New York, New York, 2017; pp. 565-566.

21. Zhang, W.; Xiao, S.; Ahn, D. U. Protein oxidation: basic principles and implications for meat quality. Critical Reviews in Food Science and Nutrition [Online] 2012, 53, Issue 11. https://www.tandfonline.com/doi/full/10.1080/10408398.2011.577540 (accessed July 11, 2018).

22. Nur, E.; Verwijs, M.; de Waart, D. R.; Schnog, J. B.; Otten, H.; Brandjes, D.P.; Biemond, B.J.; Elferink, R. P. J. O.. Increased efflux of oxidized glutathione (GSSG) causes glutathione depletion and potentially diminishes antioxidant defense in sickle erythrocytes. BBA-Molecular Basis of Disease. [Online] 2011, 1812, Issue 11. https://www.sciencedirect.com/science/article/pii/S0925443911000949?via%3Dihub (Accessed July 24, 2018).

23. Muñoz, P. M.; Ferrusola, C. O.; Vizuete, G.; Dávila, M. P.; Martinez, H. R.; Peña, F. J. Depletion of Intracellular Thiols and Increased Production of 4-Hydroxynonenal that Occur During Cryopreservation of Stallion Spermatozoa Lead to Caspase Activation, Loss of Motility, and Cell Death. Biology of Reproduction [Online] 2015, 93, pp. 1-11. https://academic.oup.com/biolreprod/article/93/6/143,%201-11/2434430 (accessed July 19, 2018).

24. Prieto-Bermejo, R.; Hernandez-Hernandez, A. The Importance of NADPH Oxidases and Redox Signaling in Angiogenesis. Antioxidants. [Online] 2017, 6(2). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5488012/ (accessed July 12, 2018).

25. Schieber, M.; Chandel, N. S. ROS Function in Redox Signaling and Oxidative Stress. Curr Biol. [Online] 2014, 24, pp. R453-R462. https://www.sciencedirect.com/science/article/pii/S0960982214003261?via%3Dihub (accessed July 29, 2018).

26. Nelson, D.L.; Cox, M.M. Lehninger: Principles of Biochemistry, 7th ed.; Publisher: Freeman, W. H. & Company, 2017; pp. 75.

27. Wong-Ekkabut, J.; Xu, Z.; Triampo, W.; Tang,I.; Tieleman, D. P.; Monticelli, L. Effect of Lipid Peroxidation on the Properties of Lipid Bilayers: A Molecular Dynamics Study. Biophys J. [Online] 2007, 93, pp. 4225-4236. https://www.ncbi.nlm.nih.gov/pubmed/17766354 (accessed July 10, 2018).

28. Ayala, A.; Muñoz, M. F.; Argüelles, S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxidative Medicine and Cellular Longevity. [Online] 2014, 2014, pp. 1-31. https://www.hindawi.com/journals/omcl/2014/360438/ (accessed July 20, 2018).

29. Salmon, T. B.; Evert, B. A.; Song, B.; Doetsch, P. W. Biological consequences of oxidative stress-induced DNA damage in Saccharomyces cerevisiae. Nucleic Acids Research. [Online] 2004, 32, pp. 3712-3723. https://academic.oup.com/nar/article/32/12/3712/2375825 (accessed July 3, 2018).

30. Shichiri, M.; Yoshida, Y.; Niki, E. Chapter 4- Unregulated Lipid Peroxidation. In Neurological Dysfunction. In Omega-3 Fatty Acids in Brain and Neurological Health. 2014; pp. 31-55.

31. Genetics Home Reference: Your Guide to Understanding Genetic Conditions. https://ghr.nlm.nih.gov/condition/glutathione-synthetase-deficiency (accessed July 20, 2018).

32. Essential Nutraceuticals. Glutathione. http://www.essentialgsh.com/glutathione.html (accessed July 23, 2018).

33. Allen, J.; Bradley, R. D. Effects of Oral Glutathione Supplementation on Systemic Oxidative Stress Biomarkers in Human Volunteers. The Journal of Alternative and Complementary Medicine. 2011, 17, pp. 827-833.