[Frontiers in Bioscience, Scholar, 9, 71-87, January 1, 2017]

Protein oxidation: an overview of metabolism of sulphur containing amino acid, cysteine

Saheem Ahmad[1], Hamda Khan[1], Uzma Shahab[2], Shahnawaz Rehman[3], Zeeshan Rafi[1,4], Mohd Yasir Khan[1], Ahsanullah Ansari[1], Zeba Siddiqui[1], Jalaluddin Mohammad Ashraf[5], Saleh M S Abdullah[5], Safia Habib[6], Moin Uddin[6]

[1]Department of Biosciences, Integral University, Lucknow-226026, India, [2]Department of Biochemistry, King George Medical University, Lucknow, India, [3]Rajiv Gandhi Center for Diabetes and Endocrinology, J.N. Medical College, Aligarh Muslim University, Aligarh-202002, India, [4]Department of Bioengineering, Integral University, Lucknow-226026, India, [5]Department of Biochemistry, Faculty of Applied Medical Sciences, Jazan University, Jazan, Saudi Arabia, [6]Department of Biochemistry, J.N. Medical College, Aligarh Muslim University, Aligarh-202002, India

TABLE OF CONTENTS

1. ABSTRACT

The available data suggest that among cellular constituents, proteins are the major target for oxidation primarily because of their quantity and high rate of interactions with ROS. Proteins are susceptible to ROS modifications of amino acid side chains which alter protein structure. Among the amino acids, Cysteine (Cys) is more prone to oxidation by ROS because of its high nucleophilic property. The reactivity of Cys with ROS is due to the presence of thiol group. In the oxidised form, Cys forms disulfide bond, which are primary covalent cross-link found in proteins, and which stabilize the native conformation of a protein. Indirect evidence suggests that thiol modifications by ROS may be involved in neurodegenerative disorders, but the significance and precise extent of the contributions are poorly understood. Here, we review the role of oxidized Cys in different pathological consequences and its biochemistry may increase the research in the discovery of new therapies. The purpose of this review is to re-examine the role and biochemistry of oxidised Cys residues.

2. INTRODUCTION TO FREE RADICAL

The biochemical reactions occurring inside the organelles and cells of the body are one of the main driving force which helps to sustain the human life. The laws of nature are such that one moves from the early years to childhood, then into old age and at last one becomes a weak human being ultimately leading to death. This ageing process is a common trait of the life cycle of all multicellular organisms (1). In 2010, an estimated 524 million people were aged 65 or older—8 percent of the world's population. The given estimation is expected to nearly triple to about 1.5 billion by 2050, representing 16 percent of the world's population (national institute of ageing, 2011). This predicted gain in life expectancy would

lead to an increase in the number of older people acquiring age-related chronic diseases of the cardiovascular, brain, and immune systems. An increase can cause failure of sovereignty, dependence and high social costs for individuals and society, and will enforce bigger workload and financial pressures on healthcare systems worldwide. The three main areas of investigation, which are interlinked and can give or delay the ageing process or age-related chronic diseases such as neurological disorder, stroke, and atherosclerosis, are; studies involving free radicals, co-factors, and antioxidants.

Free radicals can be defined as reactive chemical species having a single unpaired electron in an outer orbit (2). These highly reactive radicals in the human body can arise from junk food, cigarette smoking, alcohol, environmental and chemical pollutants, pesticides used in the field of agriculture, ozone, toxins, carcinogen, ionisation etc. These free radicals (such as $O_2^{\bullet -}$ and H_2O_2) can arise from modest leakages from the mitochondrial electron transport chains, chloroplasts and endoplasmic reticulum (3). The unstable configuration of these highly reactive species creates energy which is supposed to be released through reactions with nearby molecules, such as nucleic acids, lipids, proteins, and carbohydrates (1). The free radicals attack the fatty membrane, surrounding the cells, loses the capacity to transport nutrients, water or oxygen to the cells. The damage in chromosomes and nucleic acids by these highly reactive species which further leads the growth of irregular cells, is the initial step in cancer development (3). A free radical prefers to acquire electrons from the lipid membrane of a cell, initiating a free radical attack on the cell known as lipid peroxidation (4). Free radicals also attack the nucleic acid, comprises the genetic code inside the cell. The role of nucleic acid is to control the growth, common cell function, and moreover to restore the smashed tissues (3). Free radicals can directly attack membrane proteins and encourage lipid-protein, lipid-lipid and protein-protein cross-linking, all of which clearly have effects on membrane role.

These highly reactive radicals modify lipid, protein, DNA and cause a number of diseases (5). Two most important causes of death are cancer and atherosclerosis, which are the salient free radical consequences. The beginning and encouragement of cancer, the leading cause of death, are associated with chromosomal defects and oncogene activation (the gene responsible for causing cancer) (6). The elevated levels of ROS are the indicators of genotoxic damage in non-irradiated common tissues (7). Oxidative stress takes part in the various situations, including atherosclerosis, cardiovascular diseases, few cancers, ageing and neurological disorders. It is currently thought to take part as an important role in contribution to all ischemic diseases, inflammatory diseases, and hemochromatosis. The biological objective of the oxidative stress is the lipid, DNA and protein connected with structural perturbations and alterations in metabolic functions (5). The dissimilar types of endogenous and exogenous sources of free radical are responsible for the huge loss in the activity of the lipid, DNA and protein.

2.1. Sources and production of free radical

The occurrence of O_2 in the atmosphere is the first most important pollution events on earth. The reaction between oxygen ferrous iron results in the formation of harmful superoxides and hydroxyl radicals, which have an effect on the macromolecules. Free radicals and ROS are moreover derived from normal vital metabolic processes in the human body or from external sources such as X-rays, ozone, smoking, air pollutants, pesticides, junk foods and industrial chemicals (8). Its arrangement occurs continuously by both enzymatic and non-enzymatic reactions. Free radicals are also results from auto-oxidation and consequent inactivation of small molecules such as reduced flavins and thiols. Enzymatic reactions concerned with the development of free radicals include respiratory chain, in prostaglandin synthesis, in the cytochrome P-450 system, and in phagocytosis (9). Non-enzymatic reactions concerned in the formation of free radical involved reactions of oxygen with organic compounds as well as the reactions implicated ionisation. Some outwardly and internally generated sources of free radicals are shown in Figure 1 (5), but it is extensively produced endogenously.

2.2. Reactive oxygen species (ROS)

Formation of ROS occurs as the consequences of a number of biological processes. Elevated ROS production and/or a decreased antioxidant capacity are responsible for the harmful effects of free radicals or the oxidative stress. This pattern of ROS can encourage a number of forms of oxidative damage, including protein oxidation, and thereby influences the function of cellular processes. There are a number of processes by which ROS may be generated. These processes include NADPH oxidase pathways, aerobic respiration, nitric oxide synthesis, and during inflammation (1).

Nitric oxide (NO.) is produced by NO synthases (NOS) and can interact with ROS to form peroxynitrite (ONOO), which induces protein damage by formation of nitrotyrosine (10) NADPH oxidase was initially revealed in neutrophils, which on stimulation endure the respiratory burst, with the release of superoxide into the phagosome. This is the response catalysed by NADPH oxidase (Eq. 2), with electrons supplied by NADPH.

$$O_2 + e^- \rightarrow O_2^{\bullet -} \text{ (superoxide)} \quad \ldots (1)$$

$$2O_2 + NADPH2 \rightarrow O_2^{\bullet -} + NADP^+ + H^+ \rightarrow \quad \ldots (2)$$

Additionally, the reduction of oxygen produces hydrogen peroxide (H_2O_2). This can take place from the

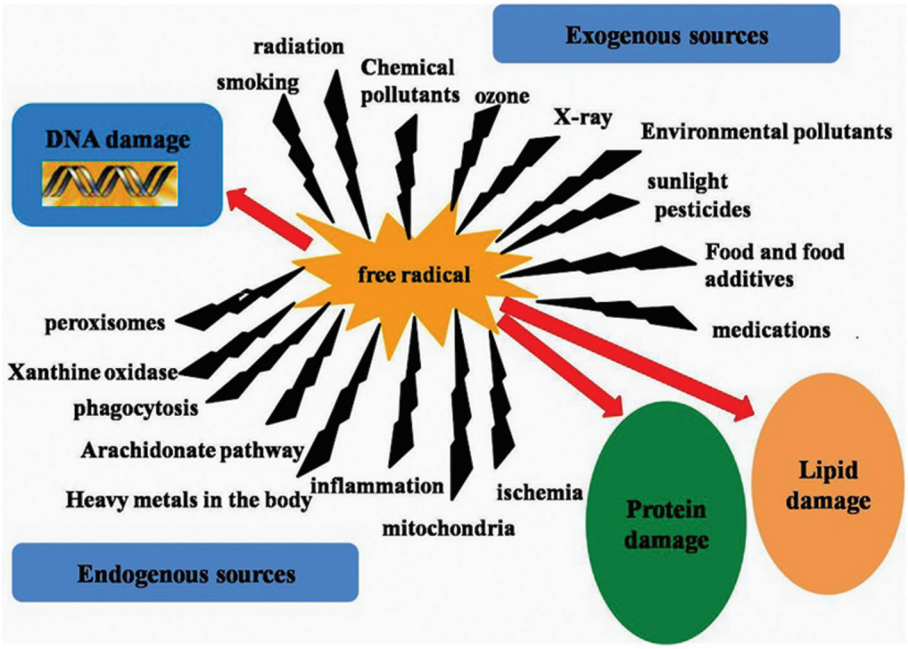

Figure 1. Exogenous and endogenous sources of free radicals causing damages to macromolecules.

dismutation of superoxide (Eq. 3), instinctively particularly at low pH:

$$2O^{-\bullet} + 2H^{+} \rightarrow H_2O_2 + O_2 \quad \ldots (3)$$

Increasing oxygen concentration very much influence the production of ROS like superoxide (O2 •−) and H_2O_2. Oxygen stress across initial tissues is compared to their space from a source of oxygen (i.e. a vessel). This dissimilarity in oxygen concentration modifies the metabolic scope of cells by allowing them to use oxidative phosphorylation to make ATP, reasonably relying on glycolysis only Allen and Balin describe this as a "metabolic gradient" that influences the development of tissues (11).

2.2.1. The superoxide anion O_2•-

It is a general reactive form of oxygen that is produced when molecular oxygen gains a single electron. Superoxide radicals are competent of attacking liable biologic targets, together with lipid, protein, and nucleic acid. O2•- is the result of the transport chain of mitochondria, or during enzymes like xanthine oxidase, NADPH-oxidase (NOX), and others or in auto-oxidation (oxidation by exposure to atmospheric oxygen and sometimes by UV irradiation) reactions. Most common characteristics of O2•- are being able to act as a mild reactant in physiological situation comparatively unreactive intermediate, and, certainly only the interaction with nitric oxide (NO) to give ONOO) is able to transform superoxide into a highly reactive intermediate; in living tissue. O2•- can be changed enzymatically, H_2O_2 by superoxide-dismutase (SOD) isoforms, or non-enzymatically. Superoxide anion ((O2•-) has thus poor quality to cross the plasma membrane.

2.2.2. Hydrogen peroxide (H_2O_2)

It is often described as "water but with one more oxygen atom", exhibits oxidising and reducing properties, depending on pH. It originates from enzymatic dismutation catalysed by superoxide dismutase (SOD) isoforms from non-enzymatic dismutation of O2•- as well as through reduction of O2. Main features of H_2O_2 include: easily diffuse across biological membranes; behave as non-radical potent oxidizing agent; it can be able to oxidize or reduce the number of inorganic ions in aqueous solution; can be usually removed by either catalase or glutathione peroxidase; when it react with O2•- then •OH is formed which is highly reactive and damaging, or in the presence of divalent metal ions like iron and copper (Cu^{2+}, Zn^{2+},) when Fe2+ (iron) is present, the reaction is also defined as Fenton's reaction (or Fe2+-catalysed Haber-Weiss reaction) (12).

2.2.3. Hydroxyl radical

Hydroxyl radical (•OH) is a three-electron reduction state of O_2 formed during Fenton or Haber-Weiss reactions or by the decomposition of peroxynitrite. Hydroxyl radical (•OH) has a very short half-life and highly reactive (13). It can reduce disulfide bonds in proteins, particularly resulting in their unfolding and scrambled refolding into irregular spatial configurations. The consequences of this effect are observed in a lot of diseases such as neurological disorders, cancer

and atherosclerosis. This effect can be prohibited by the action of non-reducing substances. Furthermore, numerous beneficial substances, usually classified as antioxidants, accept electrons and therefore it is effectual oxidants. One of the studies shows that hydroxyl radicals can be generated by ferric ions devoid of any oxidising agent. The analysis of the well-known harmful effect of inadequately chelated iron in the human can be vital in the safeguarding of human health. Though advantageous property of the immense number of phytochemicals that are endowed with hydroxyl radical scavenging and or iron chelating activities should not be considered as a proof for oxidative stress (14).

Thus, the imbalance between the production of ROS and ability of the biological systems to readily detoxify the reactive intermediates or to repair the resulting damage is the cause of oxidative stress (15). It occurs when the formation of ROS increases, or when scavenging of ROS or fixing of oxidatively modified molecules decreases (16). ROS are highly reactive, toxic oxygen moieties including hydroxyl radical, peroxyl radical, superoxide anion, and hydrogen peroxide (17) produced by cellular metabolic activities, environmental factors, such as cigarette smoke, air pollutants or pesticides. They are extremely reactive molecules because of unpaired electrons in their composition and act in response of a number of biological macromolecules in the cell, such as nucleic acid (DNA, RNA), carbohydrate, lipid, and protein, and alter their functions (18) ROS are produced as a result of normal cellular metabolism by living organisms (17).

Among the DNA, lipids and proteins, proteins are abundantly found in our body having high rate constants for reaction with many species, that is why these proteins are the major target for biological oxidants. Therefore, oxidant formation is the major consequences of protein damage both externally and within cells. The reaction between protein and ROS can take place among both the side chains and backbone, through the level of attack at definite sites dependent on numerous factors. In a number of cases, harm by ROS attack is inadequate to define residues, while with other species (e.g. hydroxyl radicals), the damage is common and unclear (19). These protein damages are mostly non-repairable, having harmful effects, with a loss (or sometimes gain) of function (e.g., enzymatic, structural, or signalling), aggregation, unfolding, fragmentation, altered interactions with other proteins, and modified turnover. The principle fate of oxidised protein is catabolism with lysosomal and proteasomal pathways, other than in a number of cases, these altered materials are badly degraded and build up within cells contributing to multiple human pathologies. (20). Hence, it is necessary to recognise the factors that control a protein's reactivity towards biological oxidant from both a bio processing and a biomedical perception.

Protein oxidation is one of the most ubiquitous forms of chemical modification. The sulphur-containing amino acids, Cys, and methionine (Met) are likely to modify by a broad range of oxidants. The protein contains many reactive residues (21). Among all the amino acids, the sulphur atoms of Cys and Met are prone to oxidation and form different oxidation states (22) Cys residues (having thiol group) purpose in the catalytic cycle of lots of enzymes, and they can form disulfide bonds that contribute to protein structure. While on the other hand, Met residues constitute an important antioxidant defence mechanism. A variety of biological oxidants readily with Met to form methionine sulfoxide (MetO), and surface exposed Met residues generate a tremendously high concentration of reactant, available as a capable biological oxidant scavenger (23).

Met residues in proteins can be readily oxidised via ROS to MetO. MetO is a capable of the physiological marker of oxidative stress and its incompetent repair by MetO reductases has been concurrent to ageing and neurodegenerative diseases (22). On the other hand, Cys is of particular concern for the reason that the thiol moiety (-SH) in the side chain of Cys makes the amino acid extremely responsive to oxidation and can form disulfide bonds with a different thiol moiety. That bond can be reduced back to the free thiol moiety (-SH) under physiological intracellular conditions. Therefore, the Cys residues exist on the protein surface are considered to be the physiological targets for ROS. This oxidative reaction has been considered to occur non-specifically, but current studies have shown the presence of extremely reactive Cys selectively oxidised by ROS. Cys residues in redox proteins, such as thioredoxin and peroxiredoxin, are highly reactive, and their functions have also been investigated from the view point of physiology and diseases (21).

To understand the correlation between oxidative stress and diseases, it is necessary to be in a position to specify the type of modification which is responsible for the oxidation of specific cellular components such as protein, lipid and DNA. Amino acid oxidation resulting from oxidative stress is related to certain diseases and ageing. The oxidative modification of free amino acids and residues in proteins has been overviewed. The purpose of this article is to re-examine the oxidised Cys residues and its role in different diseases. We will begin by covering the basics of protein oxidation. Further, we will review the oxidised amino acids and its oxidative products. Our primary focus will be on oxidised Cys residues and its effects in different diseases.

3. PROTEIN OXIDATION: A BRIEF MECHANISM

The biological target for the highly ROS is DNA, RNA, proteins, and lipids. Much of the damage is caused

by hydroxyl radicals generated from H_2O_2 via the Fenton reaction, which requires iron (or another divalent metal ion, such as copper) and a source of reducing equivalents (possibly NADH) to regenerate the metal (16).

$$O_2^{\bullet -} + H_2O_2 \xrightarrow{Fe/Cu} HO^{\bullet} + HO^{-} + O_2 \text{ Haber-Weiss reaction} \quad \text{.... (4)}$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + HO^{-} \text{ Fenton reaction} \quad \text{.... (5)}$$

At the cellular level, when proteins are exposed to ROS, modification of amino acid side chains occurs, and consequently, the protein structure is altered. This modification leads to the functional changes that disturb cellular metabolism. The observation of damaging actions of oxidised proteins and its accumulations in several pathological states such as neurodegenerative diseases, diabetes, atherosclerosis and ageing, highly increased the research in this field in the last decades of the 20th century (16).

3.1. ROS production and intracellular protein oxidation

ROS leading to protein oxidation included radical species such as superoxide ($O_2^{\bullet -}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), and non-radical species such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$). Carbonyl (CO) groups (aldehydes and ketones) are produced on protein side chains (especially of Proline, Arginine, Lysine, and Threonine) when they are oxidised. These moieties are chemically stable, which is useful for both their detection and storage. Protein carbonyl derivatives can also be generated through oxidative cleavage of proteins by either the α-amidation pathway or by oxidation of the glutamyl side chain, leading to the peptide bond structure in which the N-terminal amino acid is blocked-up by an α-ketoacyl derivative (24).

In addition, CO group may be introduced into proteins by secondary reaction of the nucleophilic side chains of Cys, His, and Lys residues, with aldehydes (4-hydroxy-2-nonenal, malondialdehyde, 2-propenal (acrolein)) produced during lipid peroxidation or with reactive carbonyl derivatives (e.g. ketoamines) generated as a result of the reaction of reducing sugars like glucose, fructose, galactose, or their oxidation products with lysine residues of proteins (glycoxidation and glycation reactions), with the eventual formation of the advanced glycation/lipoxidation end products (AGEs/ALEs), that is, glycoxidation products, such as carboxymethyllysine and pentosidine, and lipoxidation products, such as malondialdehyde-lysine and 4-hydroxy-nonenal-protein adduct (25). AGEs accumulate on serum proteins and in various tissues, particularly during ageing, diabetes, and renal failure. Elevated AGEs levels contribute to the development of diabetic and uremic complications, such as atherosclerosis, nephropathy, and retinopathy (26). It is predicted that new therapies designed to target AGEs, including AGE formation inhibitors and cross-link breakers, as well as targeting ROS using a novel highly specific antioxidants, will become part of the treatment regimen for diabetic renal disease (27).

The intracellular proteins are relatively rich in aliphatic (hydrophobic) as well as charged residues. These are recognised as a key target of oxidative change and the increase of oxidised proteins is a distinguishing feature of ageing cells. An increase in the total of oxidised proteins has been reported in several experimental ageing models, as measured by the level of intracellular protein carbonyls, or by the increase of protein-containing pigments such as ceroid bodies and lipofuscin. Intracellular protein oxidation involves different processes of oxidation such as metal catalysed oxidation, oxidation induced cleavage and amino acid oxidation. Each oxidation gives the result to the different type of oxidative products (28). For the intracellular protein oxidation, protein carbonyl is used as biomarkers of oxidative stress (29). Intracellular protein oxidation is responsible for protein misfolding, protein aggregation, post-translational modifications which further leads to different human diseases. Hence, to precede the research in the field of protein oxidation can be beneficial for the discovery of new therapies.

3.2. Metal-catalyzed oxidation

It is one of the most common mechanisms inducing protein oxidation, especially for the preface of carbonyl groups. This method requires the production of H_2O_2 and the existence of ions such as iron (Fe III) or copper (Cu II). Nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and former oxidation systems catalyse the formation of hydrogen peroxide (H_2O_2) and Fe (II) or Cu (I), derives from oxygen and from Fe (III) or Cu (II). Fe (II) and Cu (II) ions after that connect to a specific metal binding position contained by the protein and react with H_2O_2 to produce OH that then attacks the amino acid residues close to the metal binding position (30).

3.3. Oxidation-induced cleavage

The diamide pathway and α-amidation pathway is the two pathways involved in the induction of cleavage of peptide bonds in proteins by ROS. Hydroxyl radical which are the highly reactive species, generated from H_2O_2 or generated from radiolysis, reacts by means of proteins to figure out water and a carbon-centered radical (alkyl-radical such as methane, ethane, propane). This extremely reactive radical cross-link with other alkyl-radicals (e.g.: methane CH3, ethane (C_2H_5) and then a variety of protein aggregates or react with O2 to generate alkyl peroxide radical. It can then be converted

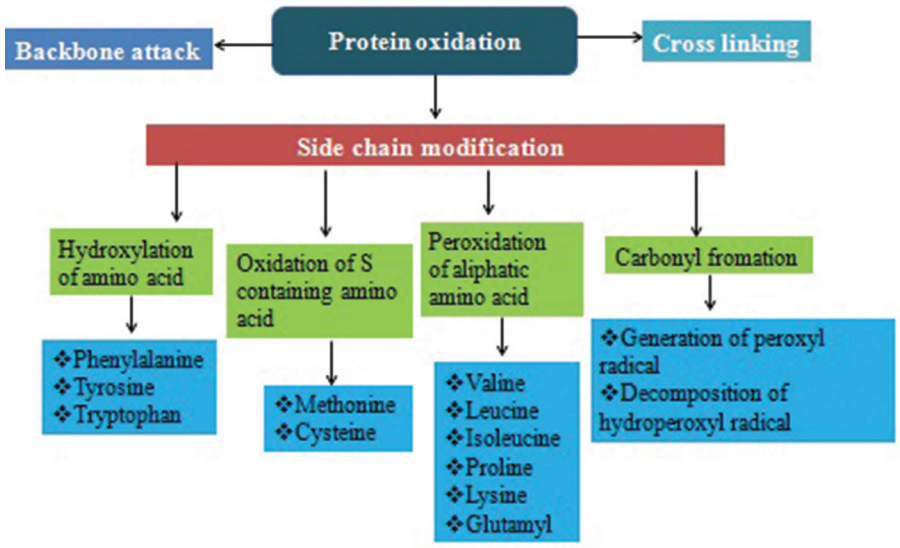

Figure 2. Protein oxidation and different types of modifications during oxidation.

throughout the action of Fe (II) or HO_2S, to alkyl peroxide. Alkyl peroxide reacts with Fe (II), HO_2S to produce an alkoxyl radical (24). The cleavage of the peptide bond present in protein can also be obtained by the reaction of the free radical, OH with the aspartyl, glutamyl, prolyl and residues of the protein chain.

3.4. Amino acid oxidation

Among the 20 amino acids of the protein, several amino acids can be able to directly modify by means of side chain reactions with ROS. Most susceptible amino acids are those having sulfhydryl groups and those with aromatic side chain groups. The condition of aromatic side-chain amino acids, including tryptophan, phenylalanine, the ROS-induced oxidation starts during an array of intermediates. For example; the oxidation of phenylalanine residues leads to the development of mono-and di-hydroxy derivatives but tryptophan residues are transformed into numerous hydroxy-derivatives, to formylkynurenine and to nitrotryptophan. Furthermore, histidine residues can be oxidised to 2-oxohistidine and 4-OH-glutamate, though tyrosine residues are converted to a dihydroxy-derivative, dopamine (DOPA), nitrotyrosine, chlorotyrosin and a dityrosine derivative. Carbonyl group can further react with α-amino group of lysine residues, which lead to the formation of intra- or inter-molecular cross-links promoted protein aggregation (31).

We are focusing on the oxidised Cys and Met and its chemistry and biochemistry in the consequences of diseases (32). During the lifetime, living organisms are constantly exposed to one or more conditions that generate highly reactive oxygen and nitrogen species (ROS/RNS) that either serve as second messengers in signal transduction or may damage protein, nucleic acid and lipid (33). As we know that alteration in signal transduction pathways can cause several chronic disorders like defects in map kinase pathway (MAPK) causes apoptosis, cancer. These alterations mostly occur through ROS. The role of ROS in normal physiological signalling, growing evidence implicates alterations in redox signalling as a contributor to many disease processes. Figure 2 showing the types of protein oxidation and the respective amino acids most susceptible to oxidation. Each amino acid gets oxidised and form different physiological oxidation product (Table 1) which are responsible for the number of diseases in human.

General mechanisms that activate the oxygen free radical-promoted oxidation of free amino acids, which forms the different oxidative products and amino acid residues of proteins are derived from radiolysis studies (5). One of the studies indicates that the most familiar pathway for the oxidation of uncomplicated aliphatic amino acids like glycine, alanine involves the hydroxyl radical-mediated concept of the formation a carbon-centered radical by hydrogen atom at the alpha-position of the amino acid and its residues in the polypeptide chain. The addition of O2 to the carbon-centered radicals leads to the development of peroxy radical derivatives, which leading decomposition for the production of ammonia (NH3) and alpha-ketoacids, or to the production of NH3, carbon-di-oxide (CO_2), and aldehydes or carboxylic acids having one less carbon atom. Since the number of carbon atoms in the amino acid is increased, hydrogen concepts at other positions in the carbon chain became more significant and lead either to the formation of hydroxy derivatives, or to amino acid cross-linked products as a result of carbon-centered radical recombination processes. Alpha-Hydrogen

Table 1. Oxidised amino acids with their different physiological oxidation product

S.No	Amino Acids	Physiological Oxidation Product
1	Phenylalanine	2,3-Dihydroxyphenylalanine, 2,-3, & 4 hydroxyphenylalanine
2	Tyrosine	Dityrosine, Nitrotyrosine, Chlortyrosine, Dihyroxyphenylalanine
3	Tryptophan	Hydroxy-& nitro tryptophan, Kynurenine
4	Histidine	2-oxohistidine, Asparagine, Aspartic acid, HNE-His
5	Methionine	Methionine sulfoxide, Methionine sulfone
6	Cysteine	Disulfide, Mixed disulfide (e.g., glutathiolation), HNE-Cys, Cystic acid
7	Valine	Hydro(pero) xides
8	Leucine	Hydro(pero) xides
9	Proline	2-Pyroolidone, 4-& 5-hydroxyproline, Pyroglutamic acid, Glutamic semialdehyde.
10	Lysine	HNE-Lys, Acrolein-Lys, Carboxymethylysin, PHA-Lys, 2-Aminoadipic semialdehyde
11	Glutamyl	Oxalic acid, Pyruvic acid
12	Arginine	Glutamic semialdehyde, Chloramines
13	Threonine	2-Amino -3- ketobutyric acid

concept plays a slight role in the oxidation of aromatic amino acids by radiolysis. In its place, the aromatic ring is the main site of attack leading to hydroxy derivatives, to ring scission, and in the case of tyrosine to the formation of Tyr-Tyr cross-linked dimers. The necessary pattern for the oxidation of amino acids by metal ion-catalyzed reaction (Fenton chemistry) is similar to the alpha-hydrogen concept pathway. But unlike the case of oxidation by radiolysis, this Fenton pathway is the most important machinery for the oxidation of all aliphatic amino acids, regardless of chain length, as well as for the oxidation of aromatic amino acids. The entire amino acid residues in proteins are the focus to get attacked by hydroxyl radicals generated by ionising radiation; though, the aromatic amino acids and sulphur-containing amino acids are most sensitive to oxidation (34). Here this review discusses the sulphur containing amino acid and its chemistry.

4. SULPHUR CONTAINING AMINO ACIDS: METHIONINE (MET) AND CYSTEINE (CYS)

Among the 20 amino acids Met and Cys are the principal sulphur-containing amino acids and the other amino acids are composed only of carbon, hydrogen, oxygen, and nitrogen atoms. In the periodic table, both sulphur and oxygen belong to the same group which is group 6^{th}; therefore, they are capable of making similar covalent linkages. One of the most important differences between oxygen and sulphur is sulphur has lower electro negativity. Indeed, oxygen is the second most electronegative element in the periodic table (group 6^{th}). This can be used in sulphur in Met; replacement of the sulphur with oxygen would effect in a much fewer hydrophobic amino acid. Among all amino acids, Cys eagerly forms disulfide linkages because of the ease with which it dissociates to form a thiolate anion. Despite the fact, serine differs from Cys only in the substitution of oxygen for the sulphur, does not eagerly make dioxide linkages as thiols (-SH) are much stronger acids than the alcohol that is why the alcohol group in serine does not dissociate at physiological pH. The substitution of oxygen for sulphur in S-adenosylmethionine would generate so potent a methylating agent (35).

While both Met and Cys play crucial roles in cell metabolism. Met is amongst the most hydrophobic of the 20 amino acids. It means that it has been mostly found in globular proteins in the interior hydrophobic core; in membrane-spanning protein domains, repeatedly found to interact with the lipid bilayer in the plasma membrane. These are liable to oxidation to MetO residues (36). Consider these Met residues as endogenous antioxidants in proteins. The catalytic activity of the enzyme has a little effect on the oxidation of these Met residues. These might be reduced to Met via means of the enzyme. Methionine sulfoxide reductase which is an enzyme plays an important role in the reduction (37). Met is the initiating amino acid in the production of these eukaryotic proteins; N-formyl methionine which serves the same function in prokaryotes. Since the most of the Met residues are later separated, it is noticeable that their role lies in the initiation of translation, not in protein formation. On the other hand, Cys plays a crucial role in protein structure by the good quality of its ability to form inter and intra-chain disulfide bond with other Cys residues. Generally, disulfide linkages are found in proteins designed for export or residence on the plasma membrane. In this review, we re-examined the chemistry of oxidised Cys residues and its role in human physiology as we know that Cys residues have a critical role in protein structure in comparison to Met. Both Cys and Met amino acids

present a fascinating focus to the nutritionist, protein chemist, and the metabolic scientist respectively. They play a vital role in protein structure, synthesis and function. Their metabolism is very important for many crucial functions (38).

4.1. Cysteine and its structural analysis

The oxidation of Cys is of particular interest from the possible connection of the sulphur compounds of the cell with the respiratory process. In its thiol form, Cys is the most reactive amino acid under physiological conditions and, is often used for adding fluorescent groups and spin labels. In the oxidised forms, Cys form disulfide bonds, which are the primary covalent cross- links found in proteins and which stabilise the native conformation of a protein. Thus accurate predictions of the oxidation state of Cys would have numerous applications, for example, in engineering when stabilising Cys or reactive thiol groups, in locating key reactive thiol groups, in enzymatic reactions or in determining topologies to aid three-dimensional structure predictions (35).

The thiol functional group present in Cys can go through a broad range of oxidative modifications and play an important role in human physiology. Cys appears to be the principal actor in redox signalling, functioning as a regulatory reversible molecular switch in addition to forming covalent cross-links that stabilize protein composition and act as a potent nucleophile in various enzyme active sites, the thiol group of Cys in separation of proteins undergoes oxidative alteration in reaction to changes in the intracellular redox situation. The chemistry of the thiol functionality and interconnected oxidation products must be taken into consideration. Along these lines, a number of selective methods to examine and enumerate discrete Cys modifications are key to understand their regulatory and pathophysiologic function (39).

4.2. Thiol functional group

The Cys side chain is considered as the most potent nucleophile of all amino-acid side chains under physiological conditions. This notable level of reactivity is due to the presence of a thiol functional group. The thiol group is a sulphur analogue of alcohol, but the smaller difference in electro negativity between the sulphur atom, and the hydrogen atom makes the S-H bond less polarized than the O-H bond, leading to a diminished propensity to form hydrogen bonds (40) In contrast, thiols is much more acidic in comparison to alcohols, and this property can be explained by the weakness of the S-H bond, and the greater likelihood that the negative charge will be distributed within sulphur 3d orbital's (41). The presence of a positively charged residue, such as lysine or arginine (42) as well as the formation of a hydrogen bond, may increase thiol acidity by 3-4 orders of magnitude. The reactivity of thiols is correlated with its pKa value (41).

In Cys, a thiolate side chain becomes a stronger nucleophile and readily reacts with oxidants and electrophilic species, although interactions with specific residues or metals can also stabilise the thiolate form. With its remarkable reactivity, the thiol group can play a key role in biological catalysis and serve as an important site for many posttranslational modifications. Post-translational modifications of proteins reported to occur in ageing include deamidation, racemization, phosphorylation, methylation, glycoxidation, and oxidation, as well as conformational changes by no chemical changes (43). Among these modifications, the oxidation is of specific concern because oxidative modifications of protein can be able to take place in any cells by ROS generated throughout oxygen metabolism most markedly in mitochondria that consume more than 90% of the oxygen that cells need. ROS is also generated in reactions catalysed by oxidative and reductive enzymes as potentially damaging by-products (44), or products that play essential physiological roles (45). The relatively low abundance of Cys, in comparison to other amino acids, combined with its remarkable nucleophilicity has made Cys the most common target for selective protein bio-conjugation (46), creating fertile ground for the development of site-specific strategies for protein modification. In this context, the thiol reacts as a soft nucleophile with alkyl and aryl halides, carbonyl, phosphoryl, and sulfonyl groups as well as with unsaturated compounds. There are a large number of reagents that selectively modify thiols, even in the presence of other strong nucleophile such as lysine or histidine. Such thiol conjugations can be further subdivided into two categories: reversible and irreversible. To date, the well-studied example of Cys modification is disulfide formation between two thiol groups and, thus it is no coincidence that one of the first strategies to detect thiols was inspired by the process of thiol-disulfide exchange (46).

As we discussed above the Cys side chain, by its high nucleophilic capability appears to be the major target of ROS/RNS in cells hence the sulphur atom of Cys may assume a wide range of oxidation states and each form exhibits a distinct chemical reactivity. In the thiolate form, sulphur undergoes oxidation to generate a sulfenic acid Figure 3, and this oxoform can be considered as a central species among thiol modifications. The sulfenic acid may be reduced to a disulfide by reaction with inter- and intra-molecular thiols or further oxidised to sulfinic acid, Figure 3) at high ROS/RNS concentrations. This sulfinic acid further leads to the formation of sulfonic acid (Figure 3) which is much stronger acid than the carboxylic acid. In some cases, the sulfenic acid leads to the formation of sulfenamide and thiosulfinate ester groups. The Cys reactivity landscape becomes more complex given that the thiolate may react with RNS and reactive sulphur species (RSS) to form S-nitrosothiol and persulfide, respectively. Moreover, depending upon the

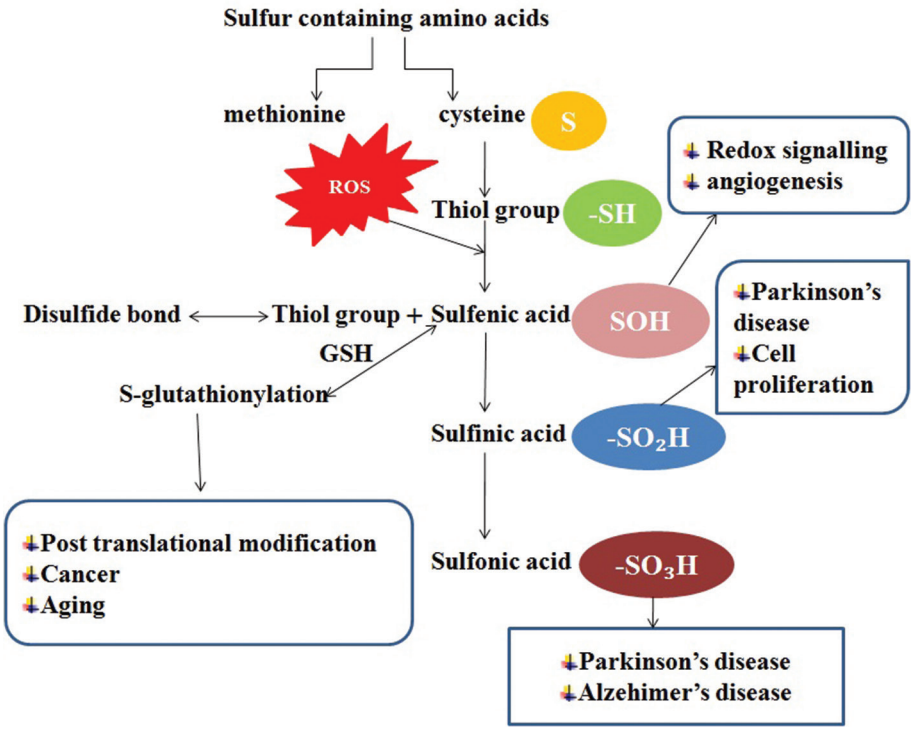

Figure 3. Cysteine oxidation and its consequences.

nature of the protein microenvironment, many of these modifications are reactive and can interconvert with one another. In recent technology, thiols are used in creating self-assembled monolayers on gold and other noble metals, protected from oxidation and other chemical effects.

4.3. Disulfide bond

Formation of disulfide bonds through a number of mechanisms is essential for the function and stability of a great number of proteins, particularly secretory protein. There exists a variety of mechanisms and pathway, which form disulfide bonds. One of the studies shows the evidence that disulfide stress is considered a specific type of oxidative stress in associated with protein γ-glutamyl Cysylation and Cysylation (47). As reactive Cys thiols interact with ROS, RNS, or chlorine species, the sulfenic acid (RSOH) occurs (Figure 3). Though stabilized in protein condition that lacks nucleophiles, such as in human serum albumin and NADPH peroxidises (48) sulfenic acids (RSOH) are highly unstable oxidation intermediates of Cys oxidation, interact rapidly within close proximity Cys to form inter- or intra-molecular disulfide bonds, creating this the major direction for oxidant-mediated disulfide bond development (49). On the other hand, sulfenic acids (RSOH) react with the small tripeptide glutathione (GSH), leading to S-glutathionylation (Figure 3). This S-glutathionylation has characteristic feature of causing several chronic disorders such as cancer, ageing.

Protein disulfide bonds are the links between the 2 Cys amino acids that form as proteins mature in the cell. Hence to review the chemistry of disulfide bonds can give benefits to the study of oxidised Cys residues in the field of diagnosis and treatment. Most of the disulfide has been generally considered to be either catalytic or structural. Structural disulfide bond stabilises the protein structure and stays unchanged throughout the life of the protein. On the other hand, catalytic bonds mediate thiol-disulfide interchange reactions in substrate proteins. The third type of disulfide bond is allosteric disulfides, have an emerging indication that a number of these bonds might be concerned in the functioning of the protein in which they exist in (50). Allosteric bonds have a configuration known as the –RHStaple. The actions of the two functional disulfides that is catalytic and allosteric are related. The redox condition of the allosteric disulfides is guarded by catalytic disulfides of the oxidoreductases (51). Catalytic bond transfer electrons between proteins, while the allosteric bonds manage the function of the protein in which they reside when they split and/or form. We know that disulfide bond formation occurs during oxidation of Cys residues. Among all the types of disulfide bond formation, allosteric disulfide bond has been less studied. Several studies show that formation of allosteric bonds during oxidised Cys residues alters the function of a protein by undergoing reversible redox transformation (52-54). By keeping these data in mind, we suggest that targeting allosteric disulfide bond in different diseases can be beneficial, which give tremendous ways

Table 2. Studies performed on different residues of cysteine in mediation of disulfide bond

S.No	Residues	Target	Study	References
1	Cys186-Cys209	Tissue Factor (TF)	Evidence for activation of tissue factor by an allosteric disulfide bond.	(78)
2	Cys-1327 and Cys-1337	Human Carbamoyl Phosphate Synthetase	Role of Cys-1327 and Cys-1337 in Redox Sensitivity and Allosteric Monitoring in Human Carbamoyl Phosphate Synthetase	(79)
3	Cys^{13}	Sar Z in S. aureus.	Crystal structures of the reduced, sulfenic acid, and mixed disulfide forms of SarZ, a redox active global regulator in Staphylococcus aureus.	(80)
4	Cys^{191}–Cys^{220}	Serine Protease	Allosteric control of βII-tryptase by a redox active disulfide bond.	(81)
5	Cys^{512}-Cys^{536}	Plasma plasminogen	Characterization of a reduced form of plasma plasminogen as the precursor for angiostatin formation.	(82)
6	Cys^{186}-Cys^{209}	Tissue factor	Evolutionary conservation of the tissue factor disulfide bonds and identification of possible oxidoreductase binding motif	(83)

for new therapies. One of the studies has been evaluate the role of an allosteric disulfide bond in cancer, which is the leading cause of death throughout the world (55). A very limited study has been performed on the role of the allosteric disulfide bond (Table 2). There is a need of some other evidence, which suggests that allosteric disulfide bond can be a good target for discovery of new therapies for different diseases such as ageing, neurological disorder, and inflammation.

4.4. Oxidised cysteine and its effect on signalling pathways

The Cys residues provide a lot of functions, such as catalysis, stabilisation of protein arrangement during metal binding, disulfides, and regulation of protein role. Cys residues also focus to many post-translational modifications. As we know that oxidative stress is the process in which organisms evolved response systems that are considered to remove ROS openly or to repair oxidative damage (56). Main players in these systems typically are proteins with redox-active amino acids, whose side-chains can directly respond with oxidants or else oxidised cellular products. Among such residues, the most frequently used is Cys (57). Reversible oxidation of Cys thiols is known to participate in redox regulation of proteins by means of the formation of sulfenic acid intermediates (RSOH), (58) inter- and intra-molecular disulfide bonds (R–S–S–R), (59) diverse disulfide bond with glutathione (R–S–SG), (60) and over oxidation to sulfinic acids ($R\text{-}SO_2H$), (61). Redox biology deals with the study of chemical reaction concerning both oxidation and reduction which results in a change in oxidation numbers of atoms integrated into the reaction. Redox signalling is a purpose that is central to all life.

Unbalanced levels of ROS are an ordinary denominator of a lot of acute and chronic degenerative diseases such as type II diabetes, atherosclerosis, renal failure, acute liver and as well as neurological disorders, including Alzheimer's disease, Parkinson's disease and stroke. Although the previous thought to induce cell death in an unspecific, necrotic way. The current study has shown that ROS may bring on cell death in an extremely regulated Caspase-independent fashion. On the other hand, research in the last years has been conventional that ROS is not only damaging the cells but at physiological level regulates a myriad of the cellular process as well as transcription regulation and cell signalling as best studied for receptor tyrosine kinase signalling (62). In many ways, ROS is preferably suitable to be signalling molecules; they are small, and can diffuse short distances; there are several mechanisms for their creation a few of which are quick and convenient and there are various mechanisms for their quick removal. Work based on the release of ROS by cells, which do not have a role in phagocytosis, and where ROS have no understandable purpose by the side of with work on host defence systems in plants has lead to the conclusion that ROS are the key signalling molecules, even though to date, their accurate form of action still wants to be elucidated. A lot of studies have indicated the role of ROS in the initiation or inhibition of cell propagation, in both activation and inhibition of apoptosis, and, at higher concentrations, in the induction of necrosis (63).

As we discussed among all the amino acids, the Cys amino acids residues are more prone to ROS. Hence, there is more probability of alteration in cell signalling through oxidised Cys (64). Several studies have been performed on oxidised Cys and its role in signalling pathways. To re-examine the role of oxidised Cys in signalling pathways can enhance the reason behind different biological consequences. The Oxidation of Cys residues of proteins is promising as a vital way of regulation of signalling pathways, mostly of protein kinase function. Signal transduction is a summary of optimistic stimuli and homeostatic negative feedback. Positive regulation of protein kinase cascades, such as those activated throughout mitogenic signalling, differentiation, and pathogenic processes such as cancer and inflammation, is mainly well studied. Reversible oxidation of proteins on Cys is potentially a resource for controlling signal transduction, since it adds an often-charged, huge moiety to the protein primary structure,

and is reversible, much like protein modifications consequential from phosphorylation (65).

Mitogen-activated protein kinase kinase 6 (MKK6) is associated with mitogen-activated protein kinase (MAPK) kinase (MAP2K) subfamily that particularly phosphorylates and activates the p38 MAPKs. One of the studies has been found that based on both biochemical and cellular assays, MKK6 was mostly susceptible to oxidation: It was inactivated by oxidation, and its kinase activity was completely restored leading treatment with a reducing agent. Thorough mechanistic studies showed that Cys 109 and 196, two of the six Cys in MKK6, formed an intramolecular disulfide bond leading oxidation that inactivated MKK6 by inhibiting its ATP binding. The two Cys involved in intramolecular disulfide arrangement are preserved in all seven members of the MAP2K family. Oxidative stress accompanies a number of human diseases, including diabetes cancer, and cardiovascular diseases, as well as neurodegenerative diseases (66). Oxidative stress is triggered by too many cellular redox state that is normally strongly regulated by oxidants, including ROS as well as RNS, and antioxidants together with thioredoxin (TrX) and GSH (66). A lot of cellular proteins are well-known to be directly regulated by the cellular redox state of the thiol group of Cys being a chief target for oxidation induced chemical modification (67). The different forms of oxidation-induced Cys modification such as sulfenic acid, sulfinic acid and sulfonic acid are known to be very much affective in the the activity and function of various redox-sensitive proteins (68).

Upon oxidation, two Cys in the active site of Trx gets oxidised, which leads to dissociation of Trx from Apoptosis signal-regulating kinase (ASK1) and successive activation of ASK1 (69). One of the research paper suggests that the way for oxidation-induced ASK1 activation may be more difficult than we initially considered for the reason that a Cys outside the kinase domain of ASK1 (i.e., Cys250) could also be directly modified by oxidation, which affects its ability to stimulate (Jun N-terminal kinase) JNK and to mediate H_2O_2 -induced apoptosis (70). Though it remains undecided how the redox state of Cys250 dictates the activity of ASK1 toward JNK. Two oblique mechanisms have also been projected to explain the oxidation-induced JNK activation: the initial involves the oxidation-induced dissociation of glutathione S-transferase pi (GSTp) from JNK (71). Comparable to the consequence of Trx on ASK1, the monomeric GSTp was also found to bind and inhibit JNK. The second indirect method involves the oxidation-induced inactivation of a JNK-specific phosphatase throughout direct alteration of the catalytic Cys (72). For kinases in group II, oxidation can regulate their activities by directly modifying key Cys in these kinases (68).

Even though one of the study data at this time evidently shows that oxidation inactivates MAP2Ks, paradoxically, a number of reports showed that oxidation could activate a variety of MAPKs (73) while others claimed that oxidation had the minimal effect on the activity of selected MAPKs (74). The similar observable fact has not before seen in other oxidation-sensitive kinases. Furthermore, this machinery is not unique to MKK6, as the researcher shows that other members of MAP2K are equally regulated by oxidation due to the presence of Cys at positions comparable to Cys109 and Cys196 of MKK6. In the future, it would be tremendously motivating and informative to investigate the biological consequence of an oxidation-resistant MKK6 within a variety of pathophysiological circumstances by knocking in the mutant gene into a needed model organism (68).

Maintenance of the cellular redox balance is vital for cell survival. A raise in reactive oxygen, nitrogen, or chlorine species can lead to oxidative stress conditions, potentially destructive DNA, lipids, and proteins. Proteins are very sensitive to oxidative modifications, mainly Met and Cys residues. The reversibility of some of these oxidative proteins modifications make them perfectly matched to take on regulatory roles in protein function. This is principally true for disulfide bond formation, which has the perspective to mediate extensively, yet fully reversible structural and functional changes, rapidly adjusting the protein's activity to the prevailing oxidant levels. Active site Cys are, by definition, extremely reactive and therefore prone to undergo oxidative modifications. One brilliant example is the active site Cys of glyceraldehyde-3-phosphate dehydrogenase (GapDH), which plays a crucial role in glycolysis. Upon exposure of GapDH to a variety of different ROS, sulfenic acid arrangement occurs at the active site Cys, followed by the arrangement of a disulfide bond with a close by Cys (75). This oxidation blocks glycolysis and appears to be answerable for the decrease in ATP levels observed in a variety of different oxidative stress treatments (76).

5. SUMMARY AND PERSPECTIVE

Oxidative modifications in protein have been traditionally considered as hallmarks of damage by oxidative stress which further leads to several human disorders like cardiovascular diseases, neurodegenerative diseases and ageing. Biological oxidants can generate a huge variety of reversible and irreversible alterations and among this alteration, the post-translational modifications can contribute to the activation of signal transduction pathways, which further mediates the toxicity of oxidants. The most appropriate reversible modifications are those arising from Cys oxidation. ROS can provoke reversible and irreversible modifications to the protein that act in diverse signalling pathways. The Cys side chain with its high nucleophilic capacity appears to be the principal target of ROS.

As we know that the brain is susceptible to oxidation. This is due to the high content of polyunsaturated

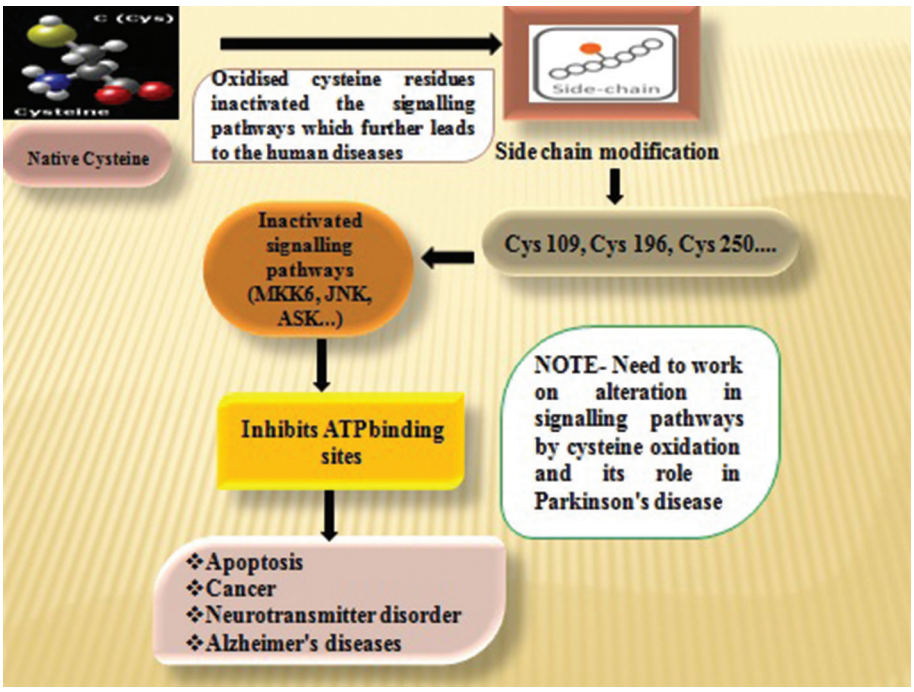

Figure 4. Summary of the review.

fatty acids, elevated rate of oxygen utilisation regional high concentration of iron, and comparatively low antioxidant capacity. Amino acids from proteins are used to make the neurotransmitter that our brain cells used for networking and communication. A most neurotransmitter is made from amino acids obtained from the protein in the food we consume. The oxidised amino acids can cause alterations in neurotransmitter which further leads to several chronic neurological disorders. As we discussed that Cys are more prone to ROS, hence this review re-examined the biochemistry of oxidised Cys, which expanded our knowledge that thiol group and disulfide bond formation is responsible for alteration in signalling pathways, which further lead to the alteration in human physiology. A few studies have been done on the role of oxidised Cys residues in the human disorders (77). In a case of neurological disorders, few scientists have done work on oxidised Cys residues in Alzheimer's diseases, (77) but no work has been done on the role of oxidised Cys residues in Parkinson's disease and ageing, which are also a neurological disorders and having mostly same symptoms of Alzheimer's disease. Therefore, by making target to the oxidised Cys residue and its role in signalling pathways in Parkinson's diseases patients may explore a way of new treatment and therapies in the field of neurological disorder.

In redox biology, the number of studies has been performed on oxidised Cys and its role in signalling pathways such as MAPK, ASK, JNK, Figure 4. These are the pathways involved in cell communication and modification which caused apoptosis, cancer, neurotransmitter disorder. Hence, it is very necessary to review the role of oxidised Cys in a mediation of these pathways in order to get the new way of treatment and medication in the field of medical sciences.

6. ACKNOWLEDGMENT

A debt of gratitude is owed to Dr Saheem Ahmad for active guidance throughout the completion of this review. The authors are also highly thankful to the funding agencies for providing financial assistance from S.E.R.B.-D.S.T (project no.) and U.P.-C.S.T. (project no.), both under young scientist category.

7. REFERENCES

1. K. Rahman: Studies on free radicals, antioxidants, and co-factors. *Clinical Interventions in Aging* 2(2): 219–236 (2007)
2. J.S. Aprioku: Pharmacology of Free Radicals and the Impact of Reactive Oxygen Species on the Testis. *J Reprod Infertil* 14(4): 158–172 (2013)
3. M. S. Hatwalne: Free radical scavengers in anaesthesiology and critical care. *Indian J Anaesth* 56(3): 227–233 (2012) DOI: 10.4103%2F0019-5049.98760. DOI: 10.4103/0019-5049.98760

4. S. Gaweł, M. Wardas, E. Niedworok, P. Wardas: Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiad Lek* 57(9-10):453-5 (2004)

5. V. Lobo, A. Pati, A. Phatak, N. Chandra: Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 4(8): 118–126 (2010)
DOI: 10.4103/0973-7847.70902

6. S. Li, Y. Wang, M. Zhao, J. Wu, S. Peng: BPIC: A novel anti-tumor lead capable of inhibiting inflammation and scavenging free radicals. *Bioorg Med Chem Lett* 25(5):1146-50 (2014)
DOI: 10.1016/j.bmcl.2014.12.013

7. P. Møller: Genotoxicity of environmental agents assessed by the alkaline comet assay. *Basic Clin Pharmacol Toxicol* 96; 1:1-42 (2005)

8. C. Sánchez-Moreno: Review: Methods Used to Evaluate the Free Radical Scavenging Activity in Foods and Biological Systems, Food Science and Technology. *International* (8)3 121-137 (2002)
DOI: 10.1106/108201302026770

9. V.O. Kaminskyy, B. Zhivotovsky: Free radicals in cross talk between autophagy and apoptosis. *Antioxid Redox Signal.* 1;21(1):86-102 (2014)
DOI: 10.1089/ars.2013.5746

10. E.W. Albrecht, C.A. Stegeman, A.T. Tiebosch, A.M. Tegzess, and H. van Goor: Expression of inducible and endothelial nitric oxide synthases, formation of peroxynitrite and reactive oxygen species in human chronic renal transplant failure. *Am J Transplant.* 2(5):448-53 (2002)
DOI: 10.1034/j.1600-6143.2002.20509.x

11. V. Cecarini, J. Gee, E. Fioretti, M. Amici, M. Angeletti, A.M. Eleuteri, J.N. Keller: Protein oxidation and cellular homeostasis: Emphasis on metabolism. *Biochimica et Biophysica Acta* 1773:93–104 (2007)
DOI: 10.1016/j.bbamcr.2006.08.039

12. W.H, Koppenol: The Haber-Weiss cycle--70 years later. *Redox Rep* 6(4):229-34 (2001)
DOI: 10.1179/135100001101536373

13. E. Cabiscol, J. Tamarit, J. Ros: Oxidative stress in bacteria and protein damage by reactive oxygen species. *Internatl Microbiol* 3:3–8 (2000)

14. B. Lipinski: Hydroxyl Radical and Its Scavengers in Health and Disease. *Oxidative Medicine and Cellular Longevity* vol 2011 pp 1-9 (2011)
DOI: 10.1155/2011/809696

15. R. Mancinelli, E. Barlocci, S. Palminiell, L. Saso: Oxidative stress and brain diseases: Biomarkers and analytical methodologies. *Indian journal of biotechnology* 10:395-403 (2011)

16. E.R. Stadtman: Protein oxidation and aging. *Science,* 257:1220–1224 (1992)
DOI: 10.1126/science.1355616

17. E. Birben, U.M. Sahiner, C. Sackesen, S. Erzurum, O. Kalayci: Oxidative stress and antioxidant defense. *World Allergy Organ J.* 5(1):9-19 (2012)
DOI: 10.1097/WOX.0b013e3182439613

18. L. Vitetta, A.W. Linnane: Endocellular regulation by free radicals and hydrogen peroxide: key determinants of the inflammatory response. *Inflammopharmacology.* 22(2):69-72 (2014)
DOI: 10.1007/s10787-014-0199-7

19. M.J. Davies: Oxidative Damage to Proteins Chemical Biology. John *Wiley & Sons*, (2012)
DOI: 10.1.002/9781119953678 (2012).

20. M.J. Davies: The oxidative environment and protein damage. *Biochem*. *Biophys.Acta*. 1703:93-109 (2005)
DOI: 10.1016/j.bbapap.2004.08.007

21. H. Miki and Y.J. Funato: Regulation of intracellular signalling through cysteine oxidation by reactive oxygen species. *Biochem.*151(3):255-61 (2012)
DOI: 10.1093/jb/mvs006

22. C. Jacob, G. I. Giles, N.M. Giles, H. Sies: Sulfur and Selenium: The Role of Oxidation State in Protein Structure and Function. *Angewandte Chemie International* 42 (39) 4742–4758 (2003)
DOI: 10.1002/anie.200300573

23. M.V. Trivedi, J.S. Laurence, T.J. Siahaan: The role of thiols and disulfides in protein chemical and physical stability. *Curr Protein Pept Sci* 10(6): 614-625 (2009)
DOI: 10.2174/138920309789630534

24. I.G. Shabalina, M. Vrbacký, A. Pecinová, A.V. Kalinovich, Z. Drahota, J. Houštěk, T. Mráček, B. Cannon, J. Nedergaard: ROS production in brown adipose tissue mitochondria: the question of UCP1-dependence. *Biochim*

Biophys Acta. 1837(12):2017-30 (2014)
DOI: 10.1016/j.bbabio.2014.04.005

25. I. Dalle-Donne, R. Rossi, D. Giustarini and A. Milzani, R. Colombo: Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta*. 329 (1-2):23-38 (2003)
DOI: 10.1016/S0009-8981(03)00003-2

26. S.G. Schwartz, M.A. Brantley, H.W. Flynn: Genetics and diabetic retinopathy. *Curr Diabetes Rev.*1;9(1):86-92
DOI: 10.2174%2F1389202911314999000 8%0A.

27. S. Vasan, P.G. Foiles, H.W. Founds: Therapeutic potential of AGE inhibitors and breakers of AGE protein cross-links. *Expert Opin Investig Drugs*. 10(11):1977-87 (2001)
DOI: 10.1517/13543784.10.11.1977

28. A. Piwowar: Perspectives on the pharmacotherapy of diseases extending with advanced oxidation protein products participation. *Postepy Hig Med Dosw* 7;68:1264-75 (2014)
DOI: 10.5604/17322693.1127949

29. I. Dalle-Donne, R. Rossi, D. Giustarini and A. Milzani, R. Colombo: Protein carbonyl groups as biomarkers of oxidative stress. *Clin Chim Acta.* 329(1-2):23-38 (2003)
DOI: 10.1016/S0009-8981(03)00003-2

30. J.D. Bridgewater, J. Lim, R.W. Vachet: Using metal-catalyzed oxidation reactions and mass spectrometry to identify amino acid residues within 10 A of the metal in Cu-binding proteins. *J Am Soc Mass Spectrom.* 17(11):1552-9 (2006)
DOI: 10.1016/j.jasms.2006.06.003

31. V. Cecarini, J. Gee, E. Fioretti, M. Amici, M. Angeletti, A.E. Maria, J.N. Keller: Protein oxidation and cellular homeostasis: Emphasis on metabolism. *Biochim Biophys Acta*.1773(2):93-104 (2006)
DOI: 10.1016/j.bbamcr.2006.08.039

32. A. Stolzing, A. Wengner, T. Grune: Degradation of oxidized extracellular proteins by microglia. *Arch Biochem Biophys*.15;400(2):171-9 (2002)
DOI: 10.1016/S0003-9861(02)00003-6

33. B.M. Riederer: Oxidation Proteomics: The Role of Thiol Modifications. *Current Proteomics,* 6:51-62 (2009)
DOI: 10.2174/157016409787847448

34. J.G. Kiselar, M.R. Chance: Future Directions of Structural Mass Spectrometry using Hydroxyl Radical Footprinting. *J Mass Spectrom* 45(12): 1373–1382 (2010)
DOI: 10.1002/jms.1808

35. A. Fiser, I. Simon: Predicting the oxidation state of cysteines by multiple sequence alignment. *Bioinformatics* 16 (3):251-256 (2000)
DOI: 10.1093/bioinformatics/16.3.251

36. R.L. Levine, L. Mosoni L, B.S. Berlett, E.R. Stadtman: Methionine residues as endogenous antioxidants in proteins. *Proc Natl Acad Sci*, 93:15036–40 (1996)
DOI: 10.1073/pnas.93.26.15036

37. J. Moskovitz: Methionine sulfoxide reductases: Ubiquitous enzymes involved in antioxidant defense, protein regulation and prevention of aging-related diseases. *Biochim Biophys Acta.* 1703:213–9 (2005)
DOI: 10.1016/j.bbapap.2004.09.003

38. B. Sperandio, P. Polard, D. S. Ehrlich, P. Renault, E. Guédon: Sulfur Amino Acid Metabolism and Its Control in *Lactococcus lactis* IL1403. *J Bacteriol* 187(11): 3762–3778 (2005)
DOI: 10.1128/JB.187.11.3762-3778.

39. D. Luo, S.W. Smith, B.D: Anderson: Kinetics and Mechanism of the Reaction of Cysteine and Hydrogen Peroxide in Aqueous Solution. *J Pharm Sci* 94: 304–316 (2004)
DOI: 10.1002/jps.20253

40. N. Wiradharma, M. Khan, L.K.Yong, C.A.E. Hauser, S.V. Seow, S. Zhang, Y.Y. Yang: The effect of thiol functional group incorporation into cationic helical peptides on antimicrobial activities and spectra. *Biomaterials* 32 (34): 9100–9108 (2011)
DOI: 10.1016/j.biomaterials.2011.08.020

41. G. Tajc, B.S. Tolbert, R. Basavappa, B.L. Miller: Direct determination of thiol pKa by isothermal titration microcalorimetry. *J Am Chem Soc* 126:10508–10509 (2004)
DOI: 10.1021/ja047929u

42. S.D. Copley, W.R.P. Novak, P.C. Babbitt: Divergence of function in the thioredoxin fold suprafamily: evidence for evolution of peroxiredoxins from a thioredoxin-like ancestor. *Biochemistry* 43: 13981–13995 (2004)
DOI: 10.1021/bi048947r

43. S. Goto, Z. Radak: Implications of oxidative damage to proteins and DNA in aging and its intervention by caloric restriction and

exercise. *Journal of Sport and Health Science* 2: 75-80 (2013)
DOI: 10.1016/j.jshs.2013.03.004

44. T. Finkel, N.J. Holbrook: Oxidants, oxidative stress and the biology of ageing. *Nature* 408: 239–247 (2000)
DOI: 10.1038/35041687

45. J. Boonstra, J.A. Post: Molecular events associated with reactive oxygen species and cell cycle progression in mammalian cells. *Gene.* 4: 1-13 (2004)
DOI: 10.1016/j.gene.2004.04.032

46. J.M. Chalker, S.B. Gunnoo, O. Boutureira, S.C. Gerstberger, M. Fernández-González, G.J.L. Bernardes, L. Griffin, H. Hailu, C.J. Schofield, B.G. Davis: Methods for converting cysteine to dehydroalanine on peptides and proteins. *Chem Sci.* 2: 1666–1676 (2011)
DOI: 10.1039/c1sc00185j

47. L.B. Poole, P.A. Karplus A, Claiborne: Protein sulfenic acids in redox signalling. *Annu. Rev. Pharmacol. Toxicol* 44: 325–347 (2004)
DOI: 10.1146/annurev.pharmtox.44.101802.121735

48. D.S. Rehder, C.R. Borges: Cysteine sulfenic Acid as an intermediate in disulfide bond formation and nonenzymatic protein folding. *Biochemistry* 49: 7748–7755 (2010)
DOI: 10.1021/bi1008694

49. M.L. Moreno, J. Escobar, A. Izquierdo-Álvarez, A. Gil, S. Pérez, J. Pereda, I. Zapico, M. Vento, L. Sabater, A. Marina, A. Martínez-Ruiz, J. Sastre: Disulfide stress: a novel type of oxidative stress in acute pancreatitis. *Free Radic Biol Med*. 70: 265-77 (2014)
DOI: 10.1016/j.freeradbiomed.2014.01.009

50. D. Butera, K.M. Cook, J. Chiu, J.S.H. Wong, P.J. Hogg: Control of blood proteins by functional disulfide bonds. *Blood.* 123: (13) (2014)
DOI: 10.1182/blood-2014-01-549816

51. P.J. Hogg: Contribution of allosteric disulfide bonds to regulation of hemostasis. *Journal of Thrombosis and Haemostasis* 1: 13-6 (2009)
DOI: 10.1111/j.1538-7836.2009.03364.x

52. I. Azimi, J.W. Wong, P.J. Hogg: Control of mature protein function by allosteric disulfide bonds. *Antioxid Redox Signal* 4: 113–126 (2011)
DOI: 10.1089/ars.2010.3620

53. K.M. Cook, P.J. Hogg: Post- translational control of protein function by disulfide bond cleavage. *Antioxid Redox Signal* 18: 1987–2015 (2013)
DOI: 10.1089/ars.2012.4807

54. M.A. Wouters, S.W. Fan, N.L, Haworth: Disulfides as redox switches: from molecular mechanisms to functional significance. *Antioxid Redox Signal*. 12: 53–91 (2010)
DOI: 10.1089/ars.2009.2510

55. P.J. Hogg: Targeting allosteric disulphide bonds in cancer. *Nature Reviews Cancer* 13: 425-43 (2013)
DOI: 10.1038/nrc3519

56. S.M. Marino, V.N. Gladyshev: Analysis and Functional Prediction of Reactive Cysteine Residues. *J Biol Chem* 287: 4419–4425 (2012)
DOI: 10.1074/jbc.R111.275578

57. C. Kumsta, U. Jakob: Redox-regulated chaperones. *Biochemistry* 48: 4666–4676 (2009)
DOI: 10.1021/bi9003556

58. H.H. Lin., L.Y. Tseng: DBCP: A web server for disulfide bonding connectivity pattern prediction without the prior knowledge of the bonding state of cysteines. *Nucleic Acids Res* 38: 503–507 (2010)
DOI: 10.1093/nar/gkq514

59. M.S. Paget, M.J, Buttner: Thiol-based regulatory switches. *Annu Rev Genet.* 37: 91–121 (2003)
DOI: 10.1146/annurev.genet.37.110801.142538

60. H.S. Chung, S.W. Wang, V. Venkatraman, C.I. Murray, J.E.Van Eyk: Cysteine Oxidative Post-translational Modifications: Emerging Regulation in the Cardiovascular System. *Circ Res* 112(2): 382–392 (2013)
DOI: 10.1161/CIRCRESAHA.112.268680

61. Z.A. Wood, E. Schroder, J. Robin Harris, L.B. Poole: Structure, mechanism and regulation of peroxiredoxins, Trends. *Biochem Sci*. 28: 32–40 (2003)
DOI: 10.1016/S0968-0004(02)00003-8

62. K. Apel, H. Hirt: Reactive oxygen species: Metabolism, Oxidative Stress, and Signal Transduction. *Annu Rev Plant Biol* 55: 373-99 (2004)
DOI: 10.1146/annurev.arplant.55.031903.141701

63. J.T. Hancock, R. Desikan, S.J, Neill:

Role of reactive oxygen species in cell signalling pathways. *Biochem Soc Trans.*29: 345-50 (2001)
DOI: 10.1042/bst0290345

64. N.M. Giles, A.B. Watts, I.G. Giles, F.H. Fry, J.A. Little child, C. Jacob C Metal: Redox Modulation of Cysteine Protein Function. *Chemistry and biology* 10: 677-693 (2003)
DOI: 10.1016/S1074-5521(03)00174-.

65. J.V. Cross, D.J. Templeton: Thiol oxidation of cell signaling proteins: Controlling an apoptotic equilibrium. *J Cell Biochem* 93: 104–111 (2004)
DOI: 10.1002/jcb.20202
66. P. Pacher, J.S. Beckman, L. Liaudet: Nitric oxide and peroxynitrite in health and disease. *Physiol Rev*. 87: 315–424 (2007)
DOI: 10.1152/physrev.00029.2006

67. L.B. Poole, K.J. Nelson: Discovering mechanisms of signaling-mediated cysteine oxidation. *Curr Opin Chem Biol*, 12: 18–24 (2008)
DOI: 10.1016/j.cbpa.2008.01.021

68. Y. Diao, W. Liu, C.C.L. Wong, X. Wang, K. Lee, P. Cheung, L. Pan, T. Xu, J. Han, J.R. Yates III, M. Zhang, Z. Wu: Oxidation-induced intramolecular disulfide bond inactivates mitogen-activated protein kinase kinase 6 by inhibiting ATP binding. *Proc Natl Acad Sci U S A* 107: 20974–20979 (2010)
DOI: 10.1073/pnas.1007225107

69. A. Matsuzawa, H. Ichijo: Redox control of cell fate by MAP kinase: physiological roles of ASK1-MAP kinase pathway in stress signalling. *Biochim Biophys Acta* 1780: 1325–1336 (2008)
DOI: 10.1016/j.bbagen.2007.12.011

70. P.J. Nadeau, S.J. Charette, J. Landry: REDOX reaction at ASK1-Cys250 is essential for activation of JNK and induction of apoptosis. *Mol Biol Cell*. 20: 3628–3637 (2009)
DOI: 10.1091/mbc.E09-03-0211

71. U. Bhattacharya, B. Halder, S. Mukhopadhyay, A.K. Giri: Role of oxidation-triggered activation of JNK and p38 MAPK in black tea polyphenols induced apoptotic death of A375 cells. *Cancer Sci* 100(10):1971-8 (2009)
DOI: 10.1111/j.1349-7006.2009.

72. H. Kamata, S. Honda, S. Maeda, L. Chang, H. Hirata and M. Karin: Reactive oxygen species promote TNF alpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatises.http://www.ncbi.nlm.nih.gov/pubmed/15766528Cell 120: 649-61 (2005)
DOI: 10.1016/j.cell.2004.12.04.

73. J.A. McCubrey, M.M. Lahair, R.A. Franklin: Reactive oxygen species-induced activation of the MAP kinase signaling pathways. *Antioxid Redox Signaling.* 8: 1775–1789 (2006)
DOI: 10.1089/ars.2006.8.1775

74. Y.M. Go, J.J. Gipp, R.T. Mulcahy, D.P. Jones: H2O2-dependent activation of GCLC-ARE4 reporter occurs by mitogen-activated protein kinase pathways without oxidation of cellular glutathione or thioredoxin-1. *J Biol Chem*. 279: 5837–5845 (2004)
DOI: 10.1074/jbc.M307547200

75. L.I. Leichert, F. Gehrke, H.V. Gudiseva, T. Blackwell, M. Ilbert, A.K. Walker, J.R. Strahler, P.C. Andrews, U. Jakob: Quantifying changes in the thiol redox proteome upon oxidative stress *in vivo*. *Proc. Natl. Acad. Sci. U.S.A* 105: 8197–8202 (2008)
DOI: 10.1073/pnas.0707723105

76. C. Colussi, M.C. Albertini, S. Coppola, S. Rovidati, F. Galli L. Ghibelli: H2O2-induced block of glycolysis as an active ADP-ribosylation reaction protecting cells from apoptosis. *FASEB J*. 14: 2266–2276 (2000)
DOI: 10.1096/fj.00-0074com

77. C.H. Chen, W. Li, R. Sultana, M.H. You, A. Kondo, K. Shahpasand, B.M. Kim, M.L. Luo, M. Nechama, Y.M. Lin, Y. Yao, T.H. Lee, X.Z. Zhou, A.M. Swomley, D.A. Butterfield, Y. Zhang, K.P. Lu: Pin1: cysteine-113 oxidation inhibits its catalytic activity and cellular function in Alzheimer's disease. *Neurobiology of disease* 76:13-23 (2015)
DOI: 10.1016/j.nbd.2014.12.027

78. V.M. Chen, J. Ahamed, H.H. Versteeg, M.C. Berndt, W. Ruf, P.J. Hogg: Evidence for activation of tissue factor by an allosteric disulfide bond. *Biochemistry.* 3;45: 12020-8 (2006)
DOI: 10.1021/bi061271a

79. E.J. Hart, S.G. Powers-Lee: Role of Cys-1327 and Cys-1337 in redox sensitivity and allosteric monitoring in human carbamoyl phosphate synthetase. *J Biol Chem.* 284: 5977-85 (2009)
DOI: 10.1074/jbc.M808702200

80. C.B. Poor, P.R. Chen, E. Duguid, P.A. Rice,

C. He: Crystal Structures of the Reduced, Sulfenic Acid, and Mixed Disulfide Forms of SarZ, a Redox Active Global Regulator in Staphylococcus aureus. *J Biol Chem*. 284: 23517–23524 (2008)
DOI: 10.1074/jbc.M109.015826

81. K.M. Cook, H.P. McNeil, P.J. Hogg: Allosteric Control of βII-Tryptase by a Redox Active Disulfide Bond. *The Journal of Biological Chemistry*. 288: 34920-34929 (2013)
DOI: 10.1074/jbc.M113.523506

82. D. Butera, K.M. Cook, J. Chiu, J.S.W. Wong, P.J. Hogg: Control of blood proteins by functional disulfide bonds. *Blood*, 2000-2007 (2014)
DOI: 10.1182/blood-2014-01-549816

83. L.G. van den Hengel, Y.W. van den Berg, P.H. Reitsma, M.H.A. Bos, H.H. Versteeg: Evolutionary conservation of the tissue factor disulfide bonds and identification of a possible oxidoreductase binding motif. *J Thromb Haemost* 10: 161–2 (2012)
DOI: 10.1111/j.1538-7836.2011.04556.x

Abbreviations: Cys, cysteine; Met, methionine; MetO, methionine sulfoxide; GSH, glutathione; MKK6, mitogen activated protein kinase kinase 6; TrX, thioredoxin; ASK1, apoptosis signal regulating kinase; JNK, jun N-terminal kinase; GapDH, glyceraldehyde-3-phophate dehydrogenase.

Key Words: Reactive oxygen species, ROS, Protein Oxidation, Sulphur containing amino acids, Cysteine, Cys, Thiol Group, Disulfide Bond, Review

Send correspondence to: Saheem Ahmad, Department of Biosciences, Integral University, Lucknow-226026, Tel: 09452677867 Fax: 915222890809, E-mail: saheem@iul.ac.in