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Protein Thiols as an Indicator of Oxidative Stress

Oksidatif Stresin Bir Göstergesi Olarak Protein Thioller

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ABSTRACT:
Thiol is an organic compound that contain sulphhydryl group that have a critical role in preventing any involvement of oxidative stress in the cell. Among the protein defensive mechanism of the body, cysteine plays an important role in preventing an oxidative damage through its thiol functional group. These defensive functions are generally considered to be carried out by the low molecular weight thiol glutathione and by cysteine residues in the active sites of proteins such as thioredoxin and peroxiredoxin. In addition, there are thiols exposed on protein surfaces that are not directly involved with protein function, although they can interact with the intracellular environment.The process of protection of the cell against an oxidative damage occur by thiol and cystein residue that has a low molecular weight. These residue are present in the active sites of a protein like, peroxiredoxin and thioredoxin. Apart from intracellular antioxidant defense mechanism by protein thiol, there are presence of thiol in outer surface of protein that are not involved with the function of protein, even though they can interact with intracellular part of the cell.
Key words: Free radicals, Thiols, antioxidants, glutathione, cysteine-SH,

ÖZET
Tiyol; hücrelerde herhangi bir oksitadif stres durumunun oluşumunu önlemede kritik bir role sahip sülfhidril grubunu içeren organik bir bileşiktir. Vücudun defansif protein mekanizmaları arasında yer alan sistein, içerdiği işlevsel tiyol grubu ile oksidatif hasarı önlemede önemli bir rol oynamaktadır. Bu defansif işlevler, genellikle tiyoredoksin ve peroredoksin gibi proteinlerin aktif bölgelerinde yer alan sistein depoları ve düşük moleküler ağırlığa sahip tiyol glutatyon aracılığıyla gerçekleştirilmelerine göre değerlendirilirler. İlaveten, tiyol direk olarak hücreler arası ortam ile iletişim halinde olabilmesine rağmen protein işlevinde direk olarak yer almayan protein yüzeylerinde yer almaktadır. Oksidatif

hasara karşı hücrenin korunması işlevi, düşük ağırlıklı tiyol ve sistein depoları ile gerçekleşmektedir. Bu depolar tiyoredoksin ve peroredoksin gibi proteinlerin aktif bölgelerinde bulunurlar. Tiyol protein aracılığıyla gerçekleşen hücreler arası antioksidant defansif mekanizmalarından başka, hücrenin hücreler arası kısımları ile iletişim halinde olan fakat tiyol protein işlevinde yer almayan hücrenin dış yüzeyinde bulunan tiyollerde mevcuttur.
Anahtar Kelimeler: Antioksidan, glutatyon,serbest radikaller, sistein-sh, tiyol

Introduction

Oxidative stress has been implicated in many numbers of human diseases by a growing body of facts. Nevertheless, cells have multiple protective mechanisms against oxidative stress and succeed in preventing cell damage to the extent that these protective mechanisms are effective. Glutathione is a peptide found in almost every cell at a high concentration. Its unique features include a) high water solubility, permitting cells to use high concentrations of the peptide, b) an amino acid constituent (cysteine) that is readily oxidized and reduced under the mild conditions required in cell metabolism, and c) an unusual peptide bond that prevents nonspecific destruction by hydrolytic enzymes that attack normal peptide bonds.

The chemistry and biology of this important peptide provide an excellent example to begin investigation on peptide and protein chemistry.The aim of this article is to systematically survey the published literature on the role of the oxidative stress as the manifestation of numerous human diseases.

Protein Thiol

Thiols contribute the major portion of the total antioxidants that are present in the body and they play an important role in defense against reactive oxygen species. Total thiols consist of intracellular and extracellular thiols either in the free form or reduced glutathione, or thiols bound to proteins. Albumin contributes as a major component of the protein bound thiols in circulation, among the thiols that are bound to proteins, this is due to higher concentration of albumin in the circulation, which binds to sufhydryl group at its cysteine-34 residue[1].

Cysteine plays a vital role in protecting the cell from oxidative damage through its thiol functional group. These defensive functions are mostly thought to be carried out by the small molecular weight thiol glutathione and by cysteine residues in the active sites of proteins such

as thioredoxin and peroxiredoxin. In summation, in that location are those exposed on protein surfaces that are not immediately involved with protein function, although they can interact with the intracellular environment. Diminished levels of thiols have been placed in several medical disorders, including chronic renal failure and other disorders linked to kidney, cardiovascular disorders, stroke and other neurological disorders, diabetes mellitus, alcoholic cirrhosis, cancer and several other disorders.

The thiols functional group plays a major function in intracellular antioxidant defences. Cysteine residues in the active sites of proteins such as thioredoxin (Trx), glutaredoxin (Grx) and peroxiredoxin (Prx) detoxify reactive oxygen species (ROS) and reactive nitrogen species and reduce oxidized protein thiols[1,2]. The low molecular weight thiol glutathione (GSH) acts in conjunction with GSH peroxidases, Grxs and glutathione S-transferases to detoxify ROS and electrophiles and to recycle oxidized protein thiols[3].

In improver to these enzymes-catalyzed reactions, thiols can also react immediately with some ROS and reactive nitrogen species; therefore, solvent-exposed thiols within cells may lead to endogenous antioxidant defences[1,4,5]. Consequently, cysteine residues exposed on the surface of proteins without a clear functional or structural role may still constitute a significant contribution to antioxidant defenses[2]. However, this possibility is not widely recognized and there is little experimental evidence to support a protective role for exposed protein thiols. One factor impeding progress is the assumption that GSH is the quantitatively dominant intracellular thiol. Although many work have recorded the higher concentration of intracellular protein thiols[2,5–8]. Little is known about the amount of exposed protein thiols within cells in comparison to GSH, or whether they are important in cellular defence. These findings indicated that the cysteine residues exposed on the surface of proteins are the dominant intracellular thiol and that they may act as a significant function in intracellular antioxidant defences.

A free radical is any molecule capable of independent (usually brief) existence that contains one or more unpaired electrons[9]. Most free radicals in biology fit within the wider category of reactive oxygen species (ROS), which include not only oxygen-containing free radicals, such as hydroxyl radical (HO·), superoxide anion radical ($O2^{\cdot}-$), and nitric oxide ($NO^{\cdot}$), but also reactive molecules that do not contain unpaired electrons, such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and peroxynitrite anion (ONOO−). The highly reactive primary

products of lipid peroxidation, lipid hydroperoxides, are formed when free radicals attack polyunsaturated fatty acids or cholesterol in membranes or lipoproteins. Instead, they can be formed by cyclooxygenase or lipoxygenase[10]. Lipid hydroperoxides function in normal physiology by regulating enzymes and redox-sensitive genes[11, 12]. However, uncontrolled lipid peroxidation can result in cellular dysfunction and impairment. Lipid peroxidation has received a great deal of attention in many medical conditions like, preeclampsia[13, 14].

A wide spectrum of ROS function as signal transducers in normal physiological condition however, their overproduction may lead to numerous human health problems[12,15,16]. Measuring of exact level of these factor in intra and extracellular environment is challenging for researchers due to instability of them in in vitro condition and many confounding factors may overlap the accuracy of result For example, lipid hydroperoxides are generated during exposure of blood and tissues to oxygen ex vivo[16]. Another problem is the lack of gold standard methods to assess oxidative stress[17, 18]. Presently there is no authentic evidence that oxidative stress is the only contributor for progression of some of the medical status. Clarity has been hampered by the deficiency of suitable animal models and unique difficulties in planning a suitable ex vivo experiment that could be reflective of the intracellular environment in response to ROS[19].

Oxidative Stress and Thiol Status

Under conditions of moderate oxidative stress, oxidation of Cysteine residues can result in the reversible formation of mixed disulfides between protein thiol groups and low–molecular-mass thiols (S-thiolation), particularly with GSH (S-glutathionylation). Protein S glutathionylation can directly alter or regulate protein function (redox regulation) and may as well have a role in protecting from irreversible (terminal) oxidation. S-glutathiolation of protein cysteine residues protects against higher oxidation states of the protein thiol, thereby preserving the reversibility of this type of change. Second, reduced protein thiols can be regenerated from their *S*-glutathiolated forms enzymatically through the action of protein disulfide isomerase, mitochondrial glutaredoxin, or thioredoxin. Protein *S*-glutathiolation has also been implicated in the control of ubiquitination, the binding of the transcription factor c-Jun to DNA, and sarcoplasmic Ca2-ATPase activity. 39S-Glutathionylated proteins accumulate under oxidative/ nitrosative stress conditions, but they can be readily reduced to free thiol groups when normal cellular redox status is recovered by glutaredoxins (toll transferases) or

reducing agents. A characteristic hallmark of many pathophysiologic conditions is a diminution in the GSH: GSSG ratio. When GSSG accumulates in cells, it can undergo disulfide exchange reactions with protein thiols, leading to their S-glutathionylation. S-Glutathionylated proteins have been investigated as potential biomarkers of oxidative/nitrosative stress in some human diseases, such as renal cell carcinoma and diabetes. Glutathionylated hemoglobin is increased in patients with type 1 and type-2 diabetes, hyperlipidemia, and uraemia associated with haemodialysis or peritoneal dialysis[20].

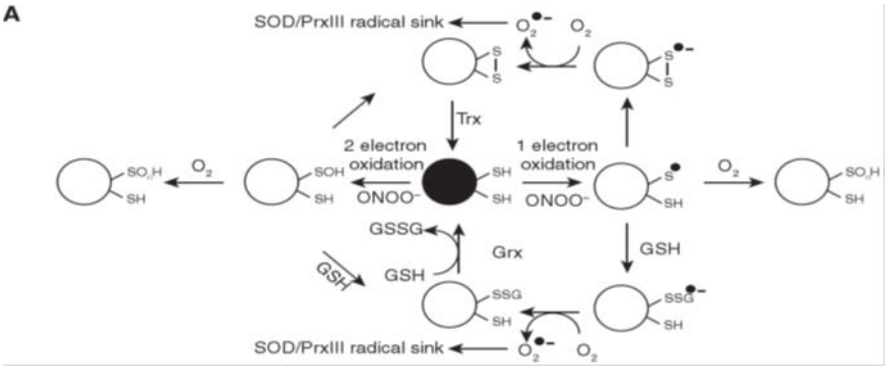
Thioredoxin, an enzyme ubiquitously expressed in endothelial cells and medial smooth muscle cells, is a major cytosolic protein thiol reductant and appears to be a target for ROS with implications for cell signalling[20]. Reversibility of the oxidation-mediated protein modification can be accomplished via the activity of another enzyme, glutaredoxin[21]. Protein thiols represent a prominent biological target for reactive nitrogen species (RNS) involved in cell signalling within the vasculature and many other tissues. *S*-nitrosation of protein cysteine residues is a motif for –NO related signalling.

Selectivity in the S-nitrosation of protein thiols represents a means for allosteric control of protein function[22]. The chemical alteration of protein thiol by ROS and RNS does not take place in isolation. Looking at its relative abundance, it is not surprising that GSH functions as a prominent coreactant for protein thiol modification in the expression of ROS and RNS. It has been estimated that proteins can scavenge the majority (50%–75%) of reactive species generated[23]. Many of this function is attributed to the thiol groups present on them. The serum levels of protein -SH in the body indicates antioxidant status and depressed degrees of protein –SH correlated positively with the increased levels of lipid peroxides and of advanced oxidation protein products (AOPPs)[24,25]. Many studies have been conducted to measure the protein thiol concentration in serum as well as the saliva of the patient with chronic disease like, oral cancer to find out the role of these antioxidant in the preventing the progression of the cancer[26,27]. Both basic and clinical research on the development of methods to assay oxidative stress are increasing and in many different types of cancer, finding a biological marker that could explain the oxidative damage is becoming a challenging issue to treat the cancer[28].

Disorders

Oxidative stress has been observed in variety of diseases. A survey has indicated that oxidative damage due to decrease in protein thiol in patient with hypercholesterolemia that might be one of the possible manifestation of ROS[29].

A substantial decline in plasma protein thiols has likewise been followed after the assisted reproduction procedures like intrauterine insemination, indicating increased oxidative stress after the procedure[30]. Oxidative stress has been implicated in the degeneration of dopaminergic neurons in the substantia nigra (SN) of Parkinson's disease (PD). An important biochemical feature of presymptomatic PD is a substantial depletion of the thiol antioxidant glutathione (GSH) in these neurons, resulting in oxidative stress, mitochondrial dysfunction, and finally result in cell death[31]. In schizophrenic patients, the amount of homocysteine in plasma was shown to be higher compared and the level of GSH, C-SH and CG-SH was decreased. This suggests that ROS and RNS may stimulate oxidative/nitrative modifications of plasma proteins in schizophrenic patients[32,33]. The Figure 1 shows the method by which exposed protein thiol, protect against oxidative stress. The three panels show the various ways of protection by protein thiol[34].



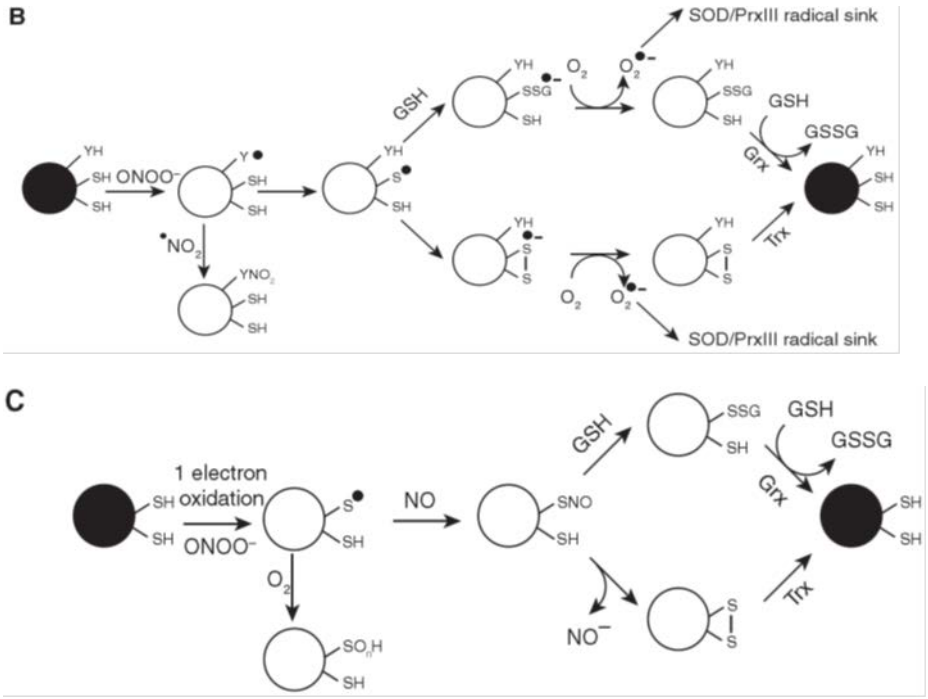


Figure 1. Schematic representation that consist of A, B, C, explains the role of protein thiol against oxidant.

Fig. 1. Modes of protection against oxidative damage by exposed protein thiols. The three panels show the various ways in which exposed protein thiols can protect against oxidative damage. (A) Modes of recycling of exposed protein thiols after oxidation. A schematic protein (shaded) is shown with two exposed thiols. Oxidation by ROS can generate a sulfenic acid (RSOH) or a thiyl radical. These can be irreversibly oxidized to higher thiol oxidation states (RSOnH). The sulfenic acid can be converted to an intramolecular disulfide, or form a mixed disulfide with GSH. The thiyl radical can form a radical anion intramolecular disulfide, or a mixed disulfide with GSH. These can lose an electron to O2 to form superoxide. The mixed disulfide thus formed can be recycled to a thiol by the action of GSH and Grx, whereas an intramolecular disulfide can be recycled by Trx. (B) Intramolecular electron transfer from a thiol to a tyrosyl radical. ROS generates a tyrosyl radical on a tyrosine residue, which is then reduced by an adjacent thiol to generate a thiyl radical. The thiyl radical can be recycled back to a thiol by the mechanisms outlined in (A). (C) Role of NO in preventing protein oxidative damage. The thiyl radical generated by ROS can react rapidly with NO to generate a S-nitrosothiol. This will decrease the extent of irreversible oxidation of the thiol. The S-nitrosothiol can then be recycled back to a disulfide as shown34.

Diabetes Mellıtus and Oxidative Stress

Free radical mediated oxidative stress has been considered in the pathogenesis of diabetes mellitus (DM) and its complications[35]. Serum protein thiols have been found to be decreased in both types of diabetes mellitus. These reductions were partly explained by metabolic, inflammatory and iron alterations[36]. Serum protein thiols have been found to be decreased in patients that are suffering from complications of type 2 diabetes mellitus[37]. There have been reports on decreased plasma thiol levels in diabetic patients[38]. Substantial reduction in plasma-SH (P-SH) levels in diabetic hemodialysis (DHD) patients compared with the level in healthy participants and DM patients. While there was no significant difference in the whole blood GSH levels between the DM patients and controls, It was significantly higher in DHD patients in comparison to the DM patients. The low P-SH level in DHD patients, but not in DM patients, suggests that dialysis is responsible for this decrease[39].

A significant increase in free iron in Fe+3 state with a decrease in protein thiols has been shown in diabetic cases under poor glycemic control[40]. The finding that thiols as facile targets of glycation and low molecular mass thiols as potent glycation inhibitors, may assist the purpose of therapeutic agents for the treatment of the complications of diabetes[41]. Raised glucose levels can cause oxidative stress in gestational diabetes (GDM) mothers. This may be due to the increased oxidative stress prevalent in GDM[42-45]. A significant increase in the erythrocytic GSH and protein thiols in GDM maternal blood when compared to controls have been observed. Cord blood levels of protein thiols were also significantly increased in GDM[46]. This may be in reply to the milieu of increased oxidative stress in case of GDM cord blood and oxidative stress in the fetus induced by GDM[45].

Human amylin (hA) is a small fibrillogenic protein that is the major constituent of pancreatic islet amyloid, which occurs in most subjects with type-2 diabetes mellitus. There is growing evidence that hA toxicity towards islet b-cells is responsible for their gradual loss of function in type-2 diabetes mellitus. Preventing hA-mediated cytotoxicity has been suggested as a route to halt the procession of this disease, although this has not yet been proven in vivo. The thiol antioxidants, N-acetyl-L-cysteine (NAC), GSH and dithiothreitol, which not merely react with ROS, but also regulate the cellular redox potential by increasing intracellular levels of GSH and/or by acting as thiol reducing agents, afford almost complete protection and inhibit the progression of hA-evoked apoptosis. These finding indicate that, in addition to the

induction of oxidative stress, hA appears to mediate cytotoxicity through signalling pathways that are sensitive to the actions of thiol antioxidants[47].

Discussion

In the present review article, we have compiled information regarding oxidative stress and the role of protein thiol as an antioxidant against oxidant. We have shown that the exposed thiols on protein surfaces are the most abundant class of thiol within the cell. The research has shown that the content of exposed protein thiols is significantly higher than that of the predominant low molecular thiol GSH in all fractions that have been investigated. These findings are an emphasis for an important role of protein thiols in intracellular redox homeostasis[7,8]. Focussing on mitochondria, it was found that the concentration of exposed protein thiols within the mitochondrial matrix was approximately 60–90 mm, which is 20–25-fold greater than that of GSH in the same compartment. Therefore, within mitochondria, the non-enzymatic reactions of thiols are dominant by those of exposed protein thiols, and not by those of GSH. Upholding a high cysteine content on the surface of proteins, where the cysteine residue is not affected in any enzymatic or structural activity, is a significant importance to the organism compared to using nonsulfur amino acids, indicating that surface cysteine residues may have a beneficial purpose[48]. A proportion of these thiols are likely to be involved in redox regulation, and may exist in local environments that favour this. Nevertheless the proportion of exposed protein thiols in this category is likely to be small; for example, < 1% of exposed mitochondrial thiols are modified by S-nitrosation[49].

It has been proposed that the high concentration of the exposed thiols within mitochondria plays a role in protecting from nonspecific damage. This can occur because of the rapid reaction of thiols with many of the damaging species present in biological systems. Furthermore, because many of these potentially protective thiol reactions occur through the thiolate form, the higher pH in the mitochondrial matrix compared to the cytosol (7.8 versus 7.2) will make these thiols approximately five-fold more reactive than elsewhere in the cell as a result of the typical pKa of protein thiols being approximately 8.5. It has been reported that reaction rate of thiols on the surface of proteins will vary widely depending on the local environment[50].

It has been stressed recently that oxidants and oxidative modifications do indeed play a major role in permanent tissue damage[51]. The damaging effects of reactive oxygen species (ROS) have likewise been

documented in the renal parenchyma, mesangial cells in culture, and on matrix components[52- 55]. Much recent interest has centered on the role of an excessive inflammatory response in atherosclerosis. Although the connection between coronary artery disease and inflammation has been well-documented in CRF, the initiating inflammatory factors remain largely obscure[56]. Reactive oxygen species generated in oxidative stress has been shown to be a sign for the activation of nuclear factor-κB (NF-κB), a major inflammatory transcription factor that activates the transcription of several inflammation mediators[57]. These inflammatory mediators can work in concert, thereby promoting atherogenesis, particularly through oxidation of LDL and leukocyte recruitment[58].

Conclusion

There are strong evidence that exposed protein thiols are of greater importance than glutathione for nonenzyme catalysed reactions of thiols with reactive oxygen and nitrogen species and with electrophiles within the cell. One such antioxidant role for exposed protein thiols may be to prevent protein oxidative damage. In the present article we reviewed the role of protein thiol as a protective mechanism for body against oxidative damage. There have been many intense studies on the intricate role of antioxidant as a defense mechanism against oxidative damage, but exploring the complex role of these defense mechanisms require an even much detail investigation.

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