

## Antioxidant Effects of Sulfur-Containing Amino Acids

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Sulfur is an essential element for the entire biological kingdom because of its incorporation into amino acids, proteins and other biomolecules. Sulfur atoms are also important in the iron-containing flavoenzymes. Unlike humans, plants can use inorganic sulfur to synthesize sulfur-containing amino acids. Therefore, plants are an important source of sulfur for humans.

Sulfur-containing compounds are found in all body cells and are indispensable for life. Some of sulfur-containing antioxidant compounds are, cysteine, methionine, taurine, glutathione, lipoic acid, mercaptopyrionylglycine, N-acetylcysteine, and the three major organosulfur compounds of garlic oil, diallylsulfide, diallyldisulfide and diallyltrisulfide.

In a comparison of the structure-function relationship among these sulfur-containing antioxidant compounds, dihydrolipoic acid (the reduced form of LA) is the most effective antioxidant. Dihydrolipoic acid contains two sulfhydryl groups and can undergo further oxidation reaction to form lipoic acid. The antioxidative activities of sulfur-containing compounds follow a general trend, the more highly reduced forms are stronger antioxidants and the number of sulfur atoms determine, at least in part, their modulatory activities on the glutathione related antioxidant enzymes. In this article, the antioxidant effects and the antioxidative activities, of sulfur-containing amino acids, are reviewed. In addition, the general antioxidant effects and the structure-function relationship of some sulfur-containing compounds are also reviewed.

**Key Words:** Cystein, methionine, taurine, lipoic acid, N-acetylcysteine, mercaptopyrionylglycine

### INTRODUCTION

Sulfur, one of the major metabolic nutrients, is a typical non-metal element. Sulfur is the third

most abundant mineral in the body, based on percentage of total body weight, and the sixth most abundant macromineral in breast milk.<sup>1,2</sup> Sulfur, a major inorganic element, is essential for the entire biological kingdom because of its incorporation into amino acids, proteins, enzymes, vitamins and other biomolecules.<sup>1</sup> Sulfur occurs in its native or elemental state and combines with iron, base metals and sulfide minerals. Unlike humans and monogastric animals, plants can use inorganic sulfur and synthesize sulfur-containing amino acids, such as, methionine and cysteine. Therefore, plants are an important source of sulfur for humans and most animals. Sulfur-containing amino acids, such as, methionine and cysteine, are more abundant in animal and cereal proteins than in legume proteins. GSH, a cysteine-containing tripeptide, is a source of dietary sulfur. Fruits and vegetables contribute over 50% of dietary GSH, while meats contribute less than 25%.<sup>1,2</sup>

Sulfur, as a part of the sulfhydryl groups, forms thioester linkages that are necessary for the activation of molecules such as acetate. Sulfur atoms are also important in the iron-containing flavoenzymes, such as, succinate dehydrogenase and NADH dehydrogenase. In addition, sulfur atoms in cysteine are responsible for the major covalent cross-links in protein structures, by the formation of disulfide bridges between two cysteine molecules, which are important in stabilizing protein conformation.<sup>1</sup>

The term <thiol> refers to compounds containing sulfur. Plasma thiols can have pro-oxidant or antioxidant actions depending on the physiological circumstances, but are generally considered antioxidant.<sup>3</sup> Because radiation causes damage to DNA through free-radical intermediates, thiols with a net-positive charge may protect

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against radiation poisoning due to concentrating in the microenvironment of DNA and scavenging free radicals.<sup>2</sup>

Sulfur-containing compounds are found in all body cells and are indispensable for life. There are a number of medical conditions for which sulfur-containing compounds could be used therapeutically. Dietary sulfur-containing amino acids or protein supplementation may be indicated for vegan athletes, children or patients with AIDS, because of increased risk of sulfur-containing amino acid deficiency in these groups.<sup>1,2,4</sup> In addition, sulfur-containing antioxidant compounds may be beneficial in a number of oxidative stress models, such as, ischemia-reperfusion injury, diabetes, cataract formation, neurodegeneration and radiation injury.<sup>1,2,5-7</sup>

The concentration level of sulfur-containing amino acids may change their antioxidative effect to a pro-oxidative effect. The ability of thiols to function as either anti- or pro-oxidants, at least in part, is determined by the type of oxidant stress and the physiological circumstances. Among plasma thiols, total cysteine is the most abundant, followed by homocysteine and GSH. These thiols are in a dynamic relationship through thiol-disulfide exchanges and redox-reactions. The albumin cysteine-34 SH-group is believed to be important for protection against oxidative stress. The antioxidant role of albumin in plasma is fortified by its cysteine-34 residue, which can directly participate in radical scavenging. Modifications of cysteine-34, resulting in loss of its electron donating SH-group, preclude its direct radical-scavenging antioxidant effects. Thiols can reduce  $\text{Cu}^{2+}$  or  $\text{Fe}^{3+}$  to  $\text{Cu}^+$  and  $\text{Fe}^{2+}$ , respectively, while being oxidized to disulfides. The reduced metal ions can then reoxidize by reaction with a superoxide. Superoxide dismutase (SOD) converts superoxide to  $\text{H}_2\text{O}_2$ . In short, these reactions produce reactive oxygen species (ROS), like superoxide,  $\text{H}_2\text{O}_2$  and hydroxyl radicals. Most of the pro-oxidant effects induced by thiol compounds have been attributed to the formation of reactive species, including superoxide,  $\text{H}_2\text{O}_2$ , thiol and hydroxyl radicals.<sup>3,8-10</sup>

Auto-oxidation of homocysteine and cysteine, in the presence of transition metal ions, results in the production of  $\text{H}_2\text{O}_2$ . Oxidative stress is generated

by the oxidation of thiols to disulfides in the presence of reducible metal ions. In addition, the SOD molecule has cysteine residues at its active site and it is possible that the disulfide in the cells interacts with these cysteine residues and produces inhibition of the enzyme activity. In some studies, pro-oxidant effects of thiol-containing compounds have been shown and it has been reported that increased levels of homocysteine and cysteine were associated with some diseases, such as, cardiovascular disease, cerebrovascular disease, renal ischemia and liver failure.<sup>8-11</sup>

### Sulfur-containing antioxidant amino acids and compounds

Free radicals are defined as molecules having an unpaired electron in the outer orbit of the electron shell. Oxygen free radicals are superoxide, hydroxyl, peroxy, alkoxy and hydroperoxy radicals. Nitrogen free radicals are nitric oxide (NO) and nitrogen dioxide. Oxygen and nitrogen free radicals can be converted to other non-radical reactive species such as  $\text{H}_2\text{O}_2$ , hypochlorous acid, hypobromous acid and peroxy-nitrite. There are two facets to free radicals in biology, they serve as signaling and regulatory molecules at physiologic levels but as highly deleterious and cytotoxic oxidants at pathologic levels. Free radicals, formed in the body and in the environment due to a number of factors, react with cellular components, causing damage. Free radicals are known to be highly reactive species that have been implicated in the pathogenesis of many diseases, such as, cancer, uremia, Alzheimer's disease, ischemia-reperfusion injury, diabetes, asthma and cataract formation.<sup>5,8,12,13</sup>

The removal of free radicals is achieved through enzymatic and non-enzymatic reactions. Effects of free radicals are controlled enzymatically by a wide range of antioxidant enzymes, such as; SOD, glutathione peroxidase (GPX) and catalase (CAT). SOD alters toxic superoxide radicals to  $\text{H}_2\text{O}_2$ . Catalase converts  $\text{H}_2\text{O}_2$  to molecular oxygen and water. GPX catalyses the conversion of  $\text{H}_2\text{O}_2$  to water. *In vivo*, there is a high degree of interaction among endogenous and exogenous antioxidants. Depending on the order of their corresponding redox potentials, it is common for one antioxidant

to regenerate another one from its oxidized species. Antioxidants *in vivo* can destroy free radicals and supplementation with antioxidants may offer increased protection against oxidative damage.<sup>5,11-13</sup>

Some of the sulfur-containing antioxidant amino acids and compounds are, cysteine, methionine, taurine, glutathione (GSH), lipoic acid (LA), N-acetylcysteine (NAC),  $\alpha$ -mercaptopyronylglycine (MPG), and the organosulfur antioxidant compounds of garlic oil, diallylsulfide (DAS), diallyldisulfide (DADS) and diallyltrisulfide (DATS).

### Cysteine

Cysteine, the limiting amino acid for GSH synthesis, is a sulfur-containing amino acid and plays an important role as an extracellular reducing agent. Cysteine is also a critical substrate for protein synthesis being the rate-limiting precursor to taurine.<sup>2,14</sup> In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine in an irreversible reaction catalyzed by the pyridoxal-5-phosphate (PLP)-containing enzyme, cystathionine- $\beta$ -synthase. Cystathionine is hydrolysed by a second PLP-containing enzyme,  $\gamma$ -cystathionase, to produce cysteine and  $\alpha$ -ketobutyrate. The excess cysteine is oxidized to taurine or inorganic sulfates or, is excreted in urine.<sup>15</sup>

Cysteine is the rate-limiting amino acid substrate for intracellular GSH synthesis. Because of its redox instability, almost all extracellular cysteine is present in the oxidized cystine state. Thus extracellular cystine is the primary source of intracellular cysteine, which is necessary for GSH synthesis.<sup>16</sup> The availability of sulfur-containing amino acids play an important role in determining the flux of cysteine between cysteine catabolism and GSH synthesis. The GSH levels in tissue are not regulated by synthesizing enzyme alone, but rather by the combination of sulfur-containing amino acids, supply and metabolism.<sup>14</sup>

Glutamate and cystine share the same amino acid transporter and compete for transport into cells. Under conditions of elevated extracellular glutamate levels, cystine transport is inhibited, resulting in depletion of cellular GSH. Depletion of GSH results in increased susceptibility of the cell to oxidative stress. Thiol antioxidants such as

cysteine, NAC and LA, protect the cells from glutamate induced cell death. At low concentrations, the primary mechanism of protection by thiol antioxidants is mediated by their proglutathione property, rather than direct scavenging of reactive oxygen.<sup>14,16</sup>

In protein-calorie malnutrition (PMC) status, oxidative stress is greatly increased in humans and rats. Thus free radical-mediated tissue damage can occur in PMC status susceptible organs, such as, lungs. In PMC status with depleted hepatic stores of GSH, cysteine administration causes restoration of the  $\gamma$ -glutamyl cysteine ligase ( $\gamma$ -GCL) activity, a rate-limiting enzyme for GSH synthesis. Cysteine or sulfur-containing amino acid supplementation is an effective method of restoring GSH status. Cysteine plays a dominant role in regulation of  $\gamma$ -GCL and cysteine sulfinate decarboxylase.<sup>14</sup>

Thiol-containing substances are normally considered as antioxidants. However, under certain circumstances, SH-containing groups exhibit double-edged effects in terms of their antioxidant/prooxidant properties. It has been shown that a high level of homocysteine and cysteine can mediate oxidative stress only in the presence of redox-active transition metal ions.<sup>11,17,18</sup> Cysteine is more susceptible to oxidation than homocysteine. Cysteine and homocysteine stimulate low density lipoprotein (LDL) oxidation at low  $\text{Cu}^{2+}$  concentrations. The prooxidant effect of cysteine toward partially oxidized LDL, may be explained by the reduction of  $\text{Cu}^{2+}$  to  $\text{Cu}^+$ , followed by the rapid breakdown of preformed lipid hydroperoxides by  $\text{Cu}^+$  ions to form lipid radicals, thereby propagating lipid peroxidation.<sup>11,17</sup> In addition, cysteine, in the presence of  $\text{Cu}^{2+}$  ions, induces the oxidative degradation of deoxyribose, the effect being metal concentration-dependent. Deoxyribose degradation is progressively enhanced by increasing  $\text{Cu}^{2+}$  concentration. Homocysteine induces minor effects in deoxyribose and is not able to induce DNA damage. The oxidative stress induced by homocysteine may be considered a minor contribution compared with other pathophysiological mechanisms leading to cardiovascular diseases in hyperhomocysteinemic patients.<sup>18</sup>

Conversely, another series of studies have shown that cysteine and homocysteine inhibited oxidation of LDL by hemin and by copper.<sup>3,17</sup>

Cysteine inhibits LDL oxidation by copper in a simple phosphate buffer. This inhibition may be due to a decrease in copper binding to LDL and the rapid conversion of  $\text{Cu}^{2+}$  to  $\text{Cu}^+$ , which may prevent the formation of the prooxidant  $\alpha$ -tocopheroxyl radical in LDL. Cystine has no prooxidant effect toward LDL because it can not reduce  $\text{Cu}^{2+}$  to  $\text{Cu}^+$ .<sup>17</sup> Plasma thiols such as reduced GSH, homocysteine and cysteine, inhibit oxidation of LDL by hemin, a physiological source of  $\text{Fe}^{3+}$ , but plasma thiols promote oxidation of LDL by free  $\text{Fe}^{3+}$ . Among plasma thiols, reduced GSH is the most effective at inhibiting hemin-dependent LDL oxidation, an intermediate effect is observed for homocysteine and cysteine is the least effective. The ability of these thiols to protect LDL from hemin-mediated oxidation is inversely related to their susceptibility to auto-oxidation. As a consequence of its lipophilic heme group, hemin integrates  $\text{Fe}^{3+}$  into the lipoprotein particle and promotes extensive lipid oxidation in both the surface layer and core lipids, to generate a highly oxidized LDL particle. Free  $\text{Fe}^{3+}$ , in contrast to hemin, is incapable of integration into the lipoprotein particle and causes oxidative damage that is mostly limited to the surface lipid layer of the LDL particle.<sup>3</sup>

In addition, cysteine has some properties characteristic of neurotransmitters; it depolarizes neuronal membranes and can be released from adult rat brain slices in a  $\text{Ca}^{2+}$ -dependent manner. Cysteine is neurotoxic if administered orally or subcutaneously to neonatal rodents. The neurotoxicity of cysteine is strongly age-dependent, and may be related to being a weak N-methyl-D-aspartate (NMDA) glutamatergic receptor agonist while higher concentrations act on both NMDA and non-NMDA receptors.<sup>19-21</sup>

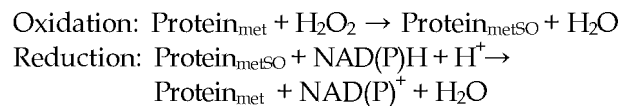
#### Methionine

Methionine, a sulfur-containing amino acid, is one of the main sources of sulfur in the body. Methionine is an essential amino acid because humans are unable to synthesize methionine from inorganic sulfur and rely on food for its supply. Methionine, an important methyl donor, is necessary for the synthesis of proteins.<sup>2</sup> Methionine is an efficient scavenger of almost all oxidizing molecules under physiological conditions, such as,

$\text{H}_2\text{O}_2$ , hydroxyl radicals, peroxyxynitrite, chloramines and hypochlorous acid.<sup>22-24</sup>

Oxidative modification of residues within proteins, may be mediated by a variety of physiologic and non-physiologic systems, including oxidases, ozone,  $\text{H}_2\text{O}_2$ , superoxide, metal-catalyzed oxidation and auto-oxidation of flavins or xenobiotics. Methionine residues may act as endogenous antioxidants. Methionine residues within a protein exhibit variability in their susceptibility to oxidation. Susceptibility generally correlates with surface exposure of the residue, although residues near the methionine can modulate its susceptibility. Surface-exposed methionine residues effectively scavenge oxidizing agents while generally preserving the biological function of the molecule. It has been shown that two surface-exposed methionine residues of interferon or three methionine residues of tissue plasminogen activation, could be oxidized without loss of biological activity. The rates of oxidation of methionine residues in some protein molecules, such as, interferon, calmodulin, human parathyroid hormone and recombinant stem cell factor exposed to  $\text{H}_2\text{O}_2$ , have been investigated.<sup>25,26</sup>

Of all the oxidative modifications of proteins, only the oxidized forms of cysteine and methionine residues can be repaired by reductases. Maintenance of the dynamic balances in biological systems, especially the balance of methionine residue oxidation and reduction, is likely to be significant for the regulation of proteins. Methionine sulfoxide reductase is capable of reducing either free methionine sulfoxide or protein-bound methionine to methionine, *in vitro* and *in vivo*.<sup>23,25</sup> Schematic equations of cyclic oxidation and reduction of methionine residues are presented;



The oxidized methionine residues are readily reduced back to methionine by methionine sulfoxide reductase, either in protein-bound or free amino acid form. Methionine residues are proposed to serve as a <last chance > antioxidant defense system to protect proteins from oxidation under conditions of oxidative stress.<sup>25,26</sup>

Homocysteine is a non-protein, sulfur-containing amino acid whose metabolism is at the intersection of two metabolic pathways; remethylation and transsulfuration. The utilization of homocysteine molecules by the two pathways may be nutritionally regulated. When excess dietary methionine was administered to human, homocysteine cycling fell below basal levels. S-adenosylmethionine, the metabolite of methionine, may be considered as an important determinant in the fate of homocysteine molecules. Intracellular methionine concentration, as a result of changes in diet, affects the rate of S-adenosylmethionine synthesis, based on the activity of methionine adenosyl transferase enzymes.<sup>2,15</sup>

Methionine metabolism is regulated by folate. 5-methyltetrahydrofolate and homocysteine are substrates, with co-factor vitamin B<sub>12</sub>, for methionine synthase in the production of endogenous methionine, which is a substrate for methionine adenosyltransferase in the daily hepatic production of 6-8  $\gamma/\delta$  of S-adenosylmethionine. S-adenosylmethionine regulates a number of methionine cycle pathways that are perturbed in alcoholic liver disease, including the generation of GSH from homocysteine. Methionine may be effective in reducing the damaging effects of alcohol and may be used in the treatment of Parkinson's disease and acute pancreatitis. Like methionine, S-adenosylmethionine is involved in numerous metabolic processes that require sulfur. In ethanol-fed baboons, S-adenosylmethionine prevents depletion of GSH levels and normalizes mitochondrial enzymes. It has been shown that administration of S-adenosylmethionine, the precursor of GSH, accelerated the clearance of ethanol and acetaldehyde in humans after ethanol intake and it has been reported that the beneficial action of S-adenosylmethionine in alcohol intoxication, is dependent on its capacity to increase the synthesis of GSH, which is actually capable of producing an adduct with acetaldehyde within the cell.<sup>2,27,28</sup>

#### *Taurine*

Taurine, a non-protein sulfur amino acid, is the most abundant free amino acid in the body and plays an important role in several essential biological processes. Taurine is derived from methio-

nine and cysteine metabolism *in vivo*, and is also readily absorbed from the diet.<sup>2,29,30</sup> Taurine is present in high concentrations in most tissues, particularly in proinflammatory cells, such as, polymorphonuclear phagocytes and in the retina. Taurine, which is present in millimolar concentrations in the retina and other membrane rich tissues, scavenges hypochlorous acid and possibly superoxide and may protect rod and other segments from structural damage induced by oxidants.<sup>2,31</sup> Taurine partially scavenges reactive oxygen species (ROS) and prevents changes in membrane permeability following oxidant injury, but does not act as a chelator of lead.<sup>31</sup>

The metabolism of perchloroethylene produces trichloroacetic acid, which conjugates with GSH either non-enzymatically or by a reaction catalyzed by GSH-S-transferase. The increased lipid peroxidation caused by perchloroethylene administration, leads to the formation of hydroperoxides, which are removed by GPX. These reactions lead to a depletion of GSH levels. Taurine and vitamin E treatment maintains GSH levels and increases the activity of GPX, the levels of SOD and CAT, and directly scavenges superoxide radicals and reduces cellular damage caused by free radicals.<sup>32</sup> The effect of dietary taurine on the toxicity of oxidized fish oil in rats has been investigated, it was found that taurine reduced the enzymatic activity of aspartate transaminase, alkaline phosphatase and alanine transaminase in the rat plasma, indicating that the liver injury caused by oxidized fish oil could be ameliorated by taurine. It was reported that the level of thiobarbituric acid reactive substances (TBARS) in the liver were reduced and the level of GSH elevated when the rats were fed with a supplement of taurine, and that taurine may play an important role in reducing the toxic effect of oxidized fish oil.<sup>33</sup> In addition, taurine depletion contributes to oxidative injury in experimental diabetic neuropathy. Dietary taurine supplementation counteracts oxidative stress, this effect of taurine is, at least in part, mediated through the ascorbate system of antioxidative defenses.<sup>34</sup>

Taurine is an essential amino acid for neonates and its intake is assured through breast milk feeding. The metabolic actions of taurine include, bile acid conjugation, detoxification, membrane

stabilization and modulation of cellular calcium levels.<sup>2,29,32</sup> Taurine decreases ventricular arrhythmia which can occur during reoxygenation after a heart attack and enables the return of normal electrical and mechanical activity. In a low calcium medium, taurine decreases tissue MDA, increases the contractile force and improves myocardial recovery from ischemic cellular injury.<sup>35</sup> In addition, taurine acts as a neurotransmitter, an osmoregulator, a regulator of calcium fluxes, a thermoregulator, anticonvulsant and cytoprotectant, and has a function as an antioxidant in most body tissues.<sup>2,29,30,36</sup>

Chlorotaurine (taurine chloramine) is formed by the direct reaction of taurine with hypochlorous acid, which is generated by the myeloperoxidase-catalyzed oxidation of H<sub>2</sub>O<sub>2</sub> during the respiratory burst. Chlorotaurine decreases both NO and tumor necrosis factor (TNF) secretion by activated macrophages in a manner that involves changes at the transcriptional and translational levels of inducible nitric oxide synthase (iNOS) and TNF expression respectively, as well as by inhibiting iNOS itself.<sup>36</sup> In addition, taurine chloramine inhibits superoxide production in a manner that is dose-dependent and reversible, and may participate in the inflammatory response by stimulating polymorphonuclear leukocytes.<sup>37</sup>

#### Glutathione

GSH, a cysteine-containing tripeptide, is synthesized from glutamate, cysteine and glycine, and is the most abundant endogenous non-protein thiol in cells.<sup>2,38</sup> GSH in the diet can be partly absorbed from the small intestine and can be synthesized de novo, so that GSH is an exogenous and endogenous antioxidant. Although the GSH-radical (GS<sup>•</sup>) formed from the oxidation of GSH is a pro-oxidant radical, GS<sup>•</sup> can react with another GS<sup>•</sup> to yield oxidized-GSH (GSSG), which is then reduced to GSH by the NADPH-dependent GSH reductase. GSSG is capable of affecting thiol-exchange reactions on thiol residues of proteins, leading to the formation of mixed disulfides and the cellular GSH pool can be regenerated from GSSG.<sup>38</sup>

GSH, a major component of the cellular antioxidant system, plays an important role in the detoxification of xenobiotic compounds and in the

antioxidation of ROS and free radicals.<sup>1</sup> GSH is a substrate for GSH-transferases and peroxidases, enzymes that catalyze the reactions for detoxification of xenobiotics and ROS.<sup>2,38</sup> Depletion of GSH, the major cellular antioxidant, results in increased vulnerability of the cell to oxidative stress. Peroxides generated by lipoxygenase and monoamino oxidase activity, may contribute to the oxidative stress that ultimately leads to the death of GSH-depleted cells. GSH can scavenge peroxynitrite with the formation of oxidized GSH (GSSG).<sup>11,16</sup> The GSH-GSH reductase system recovers, at least in part, peroxynitrite derived disulfides, but not higher sulfur oxidation state derivatives formed from peroxynitrite and H<sub>2</sub>O<sub>2</sub> reacting with isolated protein sulfhydryls.<sup>11,39</sup>

The GSH levels of tissue are not regulated by synthesizing enzymes alone, but rather by the combination of a sulfur-containing amino acid supply and metabolism. Sulfur-containing amino acids play a role in determining the flux of cysteine between cysteine catabolism and GSH synthesis. Cysteine or sulfur amino acids supplementation is an effective method of restoring GSH status.<sup>14</sup>

S-nitroso-glutathione is cleaved by the thioredoxin system to release GSH and NO. GSH also interacts with glutaredoxin and thioredoxin (thiol proteins), which play important roles in the regulation of cellular redox homeostasis. NO reacts with reduced GSH to form nitrosothiol, which can serve as a vehicle to transport NO in plasma, thereby increasing the biological half-life of NO. In addition, GSH has a mild sparing effect on vitamins C and E through its role as a reducing agent. GSH-dependent dehydroascorbate reductase regenerates ascorbate from the oxidation product, dehydroascorbate, by using GSH as an electron donor.<sup>2,11,38</sup>

Immune cell functions may be sensitive to a range of intracellular sulfhydryl compounds, including GSH and cysteine and GSH can exert an influence on immune function which is not directly related to its role as an antioxidant. GSH is able to augment the activation of cytotoxic T cells *in vivo*.<sup>4</sup>

#### Lipoic acid

LA (1,2-dithiolane-3-pentanoic acid or thioctic

acid) is a sulfur-containing cofactor, antioxidant and metal chelator. LA (the oxidized form of 6,8-dimercapto-octanoic acid) has a strained cyclic disulfide in a 1,2-dithioline ring and in its reduced form, the dihydrolipoic acid (DHHLA, 6,8-dimercaptooctanoic acid or 6,8-thioctic acid), two thiol groups per molecule are present.<sup>40-42</sup>

Humans can synthesis LA, de novo, although adequate amounts are normally found in human diets. Under normal physiological conditions, LA and DHHLA do not appear to be present in the unbound state. After dietary supplementation both forms appear in various tissues in the unbound form.<sup>43-44</sup> After LA is absorbed in the gut, it is metabolically altered in various tissues and then excreted. The liver has a high capacity for uptake and accumulation of LA or LA metabolites. Catabolic pathways involved in lipoate metabolism are largely through  $\beta$ -oxidation of the valeric acid side chain, but the carbon skeleton of the dithioline ring portion is much more resistant to alteration.<sup>45</sup>

LA is soluble in both lipid and aqueous environments and is readily absorbed from the diet, transported to cells and reduced to DHHLA. Supplemented LA enters the cell and is reduced by the cytosolic enzymes, GSH-reductase and thioredoxin reductase, and also the mitochondrial enzyme E3. The mitochondrial enzyme E3, dihydrolipoyl dehydrogenase, reduces LA to DHHLA at the expense of NADH. LA is also a substrate for the NADPH-dependent enzyme GSH-reductase.<sup>45-48</sup>

LA, a disulfide compound found naturally in mitochondria, plays a pivotal role in energy metabolism. It is involved in different multienzyme complexes such as alfa-ketoglutarate, pyruvate dehydrogenase and the glycine decarboxylase complex. LA binds acyl groups and transfers them from one part of the enzyme complex to another. In this process, LA is reduced to DHHLA, which is subsequently reoxidized by lipoamide dehydrogenase, found only in mitochondria, with the formation of NADH.<sup>40,43,49</sup>

Usually, antioxidant substances possess antioxidant properties in their reduced form. LA is unique among antioxidant molecules, because it retains protective functions in both its reduced and oxidized forms, but DHHLA is more effective

than LA in performing antioxidant functions.<sup>40</sup> Due to its unique strained cyclic disulfide structure, LA exerts significant antioxidant activities both *in vivo* and *in vitro*. LA is capable of scavenging hydroxyl radicals and hypochlorous acid, but not superoxide or peroxy radicals. DHHLA is a more potent reductant than GSH itself and reacts immediately with plasma proteins and directly reduces disulfide groups. It also prevents initiation of lipid peroxidation, scavenges hypochlorous acid, peroxy and hydroxyl radicals. Both LA and DHHLA are effective against  $H_2O_2$  and singlet oxygen.<sup>6,44,47</sup>

LA and DHHLA may prevent oxidative processes by chelating metals. Both LA and DHHLA in solution, form stable complexes with transition metals, thus they may bind and eliminate heavy metals such as  $Mn^{2+}$ ,  $Cu^{2+}$ ,  $Zn^{2+}$  and  $Pb^{2+}$  in biological systems. The chelating capacity of DHHLA is more effective than that of LA, but DHHLA may exert prooxidant actions through reduction of iron. DHHLA may increase the formation of hydroxyl radicals *in vitro* when  $Fe^{3+}$  or  $Cu^{2+}$  are included in the system and it has been shown that DHHLA has a prooxidant effect in the deoxyribose assay.<sup>6,7,40,41</sup>

LA and DHHLA are more easily reduced/oxidized compared to monothiols. Due to its redox potential, DHHLA can reduce GSSG to GSH and cystine to cysteine, but GSH and cysteine cannot reduce LA to DHHLA. LA treatment increases cellular GSH content *in vitro*, as well as *in vivo* conditions. Following LA supplementation, extracellular DHHLA reduces cystine outside the cell to cysteine. Thus DHHLA markedly improves cysteine availability within the cell, resulting in accelerated GSH synthesis.<sup>16,40,47</sup> Both LA and especially DHHLA can regenerate the well-known antioxidants, such as, vitamin C and vitamin E, GSH and coenzyme Q10 (ubiquinone), via reduction of their radical or oxidized forms, both in the membrane and aqueous phase. LA, after reduction to DHHLA, can contribute to non-enzymatic regeneration of GSH and ascorbate. Neither LA nor DHHLA alone, displayed a prominent protective effect on lipid peroxidation induced by  $Fe^{2+}$ /ascorbate, but the combination of DHHLA and GSSG did. This protective effect is due to the ability of DHHLA to reduce GSSG to GSH. In addition, DHHLA is capable of reducing ubiquinone to ubiquinol (reduced

ubiquinone) by a two-electron transfer to ubiquinone or by a one-electron transfer to ubisemiquinone anions. The high reactivity of DHLA with this potentially deleterious ubisemiquinone species, not only prevents the formation of prooxidants, but also keeps ubiquinone in its antioxidant active form.<sup>5,46,50,51</sup> LA (thioctic acid) stimulates basal glucose transport and has a positive effect on insulin-stimulated glucose uptake. Its stimulatory effect on glucose uptake is associated with intracellular redistribution of GLUT1 and GLUT4 glucose transporters and is dependent on phosphatidylinositol-3-kinase activity.<sup>52,53</sup> LA can increase glucose utilization at both normal glucose and high glucose concentrations, and reduces glycosylation of proteins and lipid peroxidation in red blood cells exposed to high glucose.<sup>41</sup>

The LA / DHLA redox couple approaches the ideal and can be considered the universal antioxidant. LA administration has been shown to be beneficial in a number of oxidative stress models, such as, ischemia-reperfusion injury, diabetes and diabetic neuropathy, neurodegeneration, radiation injury and HIV activation.<sup>2,5,7,45</sup>

#### *N-acetylcysteine*

NAC is a derivative of the sulfur-containing amino acid cysteine and is an intermediary in the conversion of cysteine to GSH. Made endogenously and found in foods, NAC has sulfhydryl groups that can scavenge free radicals.<sup>2,54</sup> NAC is readily hydrolyzed to cysteine and is able to expand natural antioxidant defenses by increasing intracellular reduced GSH concentration.<sup>54,55</sup> Thiol supplementation can maintain tissue redox balance. NAC, the cysteine delivery compound, indirectly replenishes GSH through deacetylation to cysteine and prevents oxidative damage by the scavenging of ROS. Also, NAC can prevent radiation-induced DNA breaks and act as a radioprotectant against many aspects of oxidative damage.<sup>2,56</sup> Besides its function as a cysteine donor for neuronal GSH synthesis, NAC has additional positive effects on neuronal cells as an antioxidant and in prevention of apoptotic cell death. Apoptosis, a form of active cell death, plays an important role in the development and regulation of the immune system. Oxidative stress is one of the several stimuli that induces apoptosis

in cells.<sup>47,57</sup>

NAC can reduce peroxynitrite formation by reducing nitrite production. This effect may be dependent on a direct reaction of the NAC thiol group and NO, thus producing a nitrothiol compound. The formation of these nitrothiol compounds can preserve and accumulate NO in a biologically active form.<sup>55</sup> In normotensive and spontaneously hypertensive rats, NAC, a thiol-containing compound and a powerful antioxidant, can increase the endothelium-dependent relaxation in mesenteric arteries and the aorta, and enhance the hypotensive effect of acetylcholine, through a NO-dependent mechanism.<sup>58</sup> NAC directly eliminates hydroxyl radicals and increases the nitric-oxidase-system-dependent coronary flow. NAC acts as an oxygen radical scavenger during cardiopulmonary surgery and also stabilizes neutrophils in relation to their oxidative response to the bypass.<sup>54</sup>

Originally, NAC was used to liquefy mucus in bronchi, and is also the antidote for paracetamol poisoning. Oral NAC administration leads to an increase in intracellular cysteine and GSH levels. IV NAC administration is preferable for paracetamol poisoning.<sup>2,54,56</sup> In addition, NAC administration has been reported to be beneficial in a number of oxidative stress models, such as, systemic sclerosis, HIV infection, hypertension, cardiopulmonary bypass, radiation injury and ischemia-reperfusion injury.<sup>4,54-56</sup>

#### *Mercaptopropionylglycine*

MPG, a condensation product formed by peptide linkages between thiolactic acid and glycine, is capable of liberating the-SH groups which have an important physiological function in the body.<sup>59</sup> MPG is capable of scavenging generated free radicals in the intracellular space and in the mitochondria of the reperfused heart.<sup>60</sup> MPG may cause an increase of high energy phosphates during reperfusion, by improving mitochondrial oxidative phosphorylation in the isolated perfused rat heart.<sup>61</sup> Thus the cardioprotective effect of MPG is considered to be due to prevention of free radicals. In addition, MPG has a cardioprotective effect against ischemia-reperfusion-induced contractile dysfunction. Both attenuation of sodium overload and preservation of the mitochondrial



function, may largely contribute to cardioprotection by MPG in the ischemic-reperfused heart.<sup>60</sup> MPG at relatively low and non-toxic concentrations can markedly inhibit the oxidation of tissue sulfhydryls, soluble protein and lipids, associated with ischemia-reperfusion injury of the lung. The oxidative stress on the lung tissue components results in a decrease in the sulfhydryl content of end ischemic lungs. MPG pretreatment maintains the normal sulfhydryl level in ischemic lungs.<sup>62</sup> Hepatotropic detoxification brought about by MPG, could in part, be attributed to its high redox potential. MPG is a powerful superoxide synthesis inhibitor and it may have a significant protective effect on the liver parenchyma when submitted to normothermic ischemia-reperfusion processes.<sup>59,63</sup>

MPG has a protective effect against the reactive metabolite of acetaminophen. MPG reduces the acetaminophen-induced fall in GSH levels in hepatocytes, and also decreases the covalent binding of the acetaminophen reactive metabolite to cellular protein.<sup>64</sup> MPG brings about a decline in the recovery of liver weight and the expression of *c-myc* and *c-fos* proto-oncogenes in partially hepatectomized rat liver, and down-regulates cell proliferation in regenerating liver. For this reason, MPG may find a use in cancer treatment.<sup>59</sup> In addition, MPG has been reported to be effective in a number of conditions, such as, suppression of puerperal lactation, prophylaxis of cysteine nephrolithiasis, certain hepatic disorders and radiation hazards.<sup>59,60,65-67</sup>

#### Comparison of the antioxidative activities of some sulfur-containing compounds

The antioxidative activity has mainly been designated as the peroxide value of, oxidation products from and the oxidation rate of co-existing lipids. The antioxidant capacity of a compound occurs through different mechanisms, such as, metal chelation, activated oxygen species scavenging, recycling of other antioxidants or repair of damaged molecules induced by oxidative stress. An ideal antioxidant would fulfil all these mechanisms. The LA/DHLA redox couple approaches the ideal and can be considered the universal antioxidant.<sup>64,68</sup> Sulfur forms thioester linkages

and the sulfur atoms in cysteine are responsible for the major covalent cross-links in protein structures. Oxidation of protein sulfhydryl and sulfur-containing cofactors, such as, coenzyme A, lipoic acid and thioredoxine, can perturb integrated metabolic pathways and membrane-linked functions that participate in essential metabolic and biosynthetic processes. Oxidation of low molecular weight sulfhydryls, such as, cysteine and GSH, leads to depletion of one of the most important intra-and-extracellular scavenging mechanisms serving as a defense against free radical-mediated damage.<sup>39</sup> Interconversions between disulfide and sulfhydryl groups, in oxidation-reduction reactions, are used to eliminate H<sub>2</sub>O<sub>2</sub> from the cell before it can cause cellular destruction. These interconversions occur as the sulfur-containing compound GSH is reduced and oxidized.<sup>1</sup> Depletion of GSH, the major intracellular antioxidant, results in an increased vulnerability of the cell to oxidative stress. Cysteine or cysteine-donor compounds, such as, NAC supplementation are an effective method of restoring GSH status.<sup>16,47</sup>

The oxidation of thiols and disulfides with superoxides produce sulfinates and sulfonates. Sulfides such as cysteine and oxidized GSH show larger constants than neutral amino acids. Among sulfur-containing amino acids, the oxidation rate constant for cysteine is significantly larger than those for the other thiols. According to the results of some studies, cysteine, cystine and GSH are effective antioxidants, while asparagine, alanine, glutamic acid, leucine, lysine and serine are less effective.<sup>68</sup>

The total antioxidant activities of chemical compounds cannot be evaluated by any single method because the antioxidant capacity of a compound occurs through different mechanisms, such as, activated oxygen species scavenging and metal chelation. Several methods can be used to test the antioxidant activities of various chemical compounds. Each of these assays is based on one feature of antioxidant activity, such as, the ability to scavenge free radicals or to inhibit lipid peroxidation or to prevent DNA nitration induced by nitryl chloride (NO<sub>2</sub>CL).<sup>40,69</sup>

In comparison to the structure-function relationship among sulfur-containing antioxidant com-

pounds, DHLA is the most effective in preventing DNA nitration induced by  $\text{NO}_2\text{CL}$ , NAC and folic acid are the next most effective. This might be due to the fact that DHLA contains two sulfhydryl groups and that it can undergo further oxidation, reacting to form LA.<sup>7,70</sup> LA and DHLA are more easily reduced/oxidized compared to monothiols. Due to its redox potential, DHLA can reduce GSSG to GSH, and cystine to cysteine. DHLA is a good antioxidant and LA is a moderate antioxidant.<sup>40,49</sup>

The oxidation states of sulfur atoms contribute to the difference in their antioxidative ability. Methionine is not very effective in preventing DNA nitration, induced by  $\text{NO}_2\text{CL}$ , which might be due to the primary amino group. N-acetylmethionine is a better antioxidant than methionine and taurine is the worst antioxidant at preventing  $\text{NO}_2\text{CL}$ -dependent DNA nitration, among the sulfur-containing antioxidant compounds.<sup>70-72</sup> The antioxidative activities of sulfur-containing compounds, against  $\text{NO}_2\text{CL}$ -induced DNA nitration, follow a general trend in that the more highly reduced forms are stronger antioxidants. The most highly reduced sulfhydryls are the best antioxidants, with the exception of GSH.<sup>7,49</sup> Although GSH also contains free sulfhydryls, its 50% inhibiting value is much higher than that of DHLA and NAC. The oxidized form, GSSG has a medium antioxidative activity, suggesting that further oxidation takes place.<sup>39,70</sup>

Garlic (*Allium sativum* L) has been widely used as both a folk medicine and a spice for thousands of years. Several studies have shown that garlic and its constituents have antioxidant activity, tumor-inhibitory action, anti-aging action, suppression of platelet aggregation, bactericidal and fungicidal properties.<sup>73-76</sup>

One of the most important biochemical properties of garlic is its antioxidant potential. Garlic being a strong antioxidant, exhibits antioxidant action by increasing the levels of cellular antioxidant enzymes, such as, superoxide dismutase, catalase, GSH-peroxidase and scavenging reactive oxygen species.<sup>76-78</sup> Several studies have shown that garlic contains some antioxidative compounds such as allicin, S-allylcysteine, diallylsulfide (DAS), diallyldisulfide (DADS) and diallyltrisulfide (DATS).<sup>73,78,79</sup> The stability of allicin, DADS

and DATS, was analyzed by High Performance Liquid Chromatography and it has been reported that DADS was the most stable antioxidant of these sulfur-containing compounds of garlic. In addition, the synergistic effect of  $\alpha$ -tocopherol and L- ascorbyl palmitate with the antioxidative compounds of garlic, such as, allicin, DADS and DATS, on the antioxidative process, was examined. It was reported that the greatest synergistic effect was obtained with the addition of both L- ascorbyl palmitate and  $\alpha$ -tocopherol to DADS.<sup>73</sup>

Among the organosulfur antioxidant compounds of garlic oil, DAS, DADS and DATS, are the three major components. In a comparison of the structure-function relationship among these compounds, DATS followed by DADS and then by DAS, had the greatest effect, on either the induction or inhibition of GSH-related antioxidant enzymes. Higher enzyme activity prevents the generation and accumulation of a variety of ROS and produces better protection against oxidative damage. The effectiveness of these compounds relates the number of sulfur atoms. The number of sulfur atoms determine, at least in part, their modulatory activities on the GSH related antioxidant enzymes.<sup>80</sup>

The number of sulfur atoms and allyl groups in an organosulfide molecule are important determinants of organ specificity and chemopreventive efficacy. In a comparison of the structure-activity relationship between DADS and DAS; DADS, which contains disulfide as well as allyl groups, is more potent at inhibiting benzo(a)pyrene-induced forestomach neoplasia than DAS, which contains a monosulfide.<sup>80,81</sup> In addition, it has been reported that the magnitude of the antimicrobial activity of the four diallyl sulphides in garlic followed the order diallyl tetrasulphide > DATS > DADS > DAS and that disulfide bonds are an important factor in determining the antimicrobial capabilities of these sulphides.<sup>74</sup>

In conclusion, the total antioxidant activity of a chemical compound cannot be evaluated by any single method, both the number of sulfur atoms and the oxidation states of sulfur atoms in sulfur-containing compounds can effect, at least in part, their antioxidative ability.

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