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REVIEW

BENEFICIAL AND HARMFUL EFFECTS OF THIOLS

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Biothiols are extraordinarily efficient antioxidants protecting the cells against consequences of damage induced by free radicals, due to their ability to react with the latter. In such antioxidant reactions, thiols undergo one-electron oxidation with the formation of thiyl radicals. For this reason, attention has been focused mostly on antioxidant properties of thiols. Considerably less attention has been paid to thiyl radicals ($RS^{\bullet}$) formed simultaneously in these reactions. However, protective and repairing efficacy of thiols depends not only on their capacity to detoxify free radicals but also on chemical character and reactivity of the formed thiyl radical. Furthermore, quick and efficient removal of $RS^{\bullet}$ radical leads to a disturbance in balanced state of antioxidant reaction, which effectively increases repairing capacity. Dangerous thiyl radicals, which can cause peroxidative injury, should immediately undergo regenerative reduction to thiols. Under physiological conditions, thiyl radicals can react with thiolate anion yielding disulfide radical anion $(RSSR)^{\bullet-}$ as an intermediate and finally disulfides and superoxide radical anion ($O_2^{\bullet-}$), which is next inactivated in the reaction catalyzed by superoxide dismutase (SOD). Thiyl radicals can also be reduced to thiols by reacting with ascorbate with the formation of low-activity ascorbyl radical, that subsequently enters disproportiation reaction.

Key words: *thiols, thiyl radicals, reactive oxygen species, glutathione*

An antioxidant is a substance, which is able to protect a substrate, susceptible to oxidation, from peroxidative injury, being itself present at fairly low concentrations in relation to the substrate. Biological antioxidants comprise all compounds that, at low concentrations, protect cellular lipids, proteins and nucleic acids from peroxidative damage. Biothiols play a significant biological role among these compounds due to their strong reductive ability and capacity to react with free radicals.

Types and formation of reactive oxygen species

Free radicals are atoms or groups of atoms bearing an unpaired electron, thereby being a cause of a damage to tissues and organs, leading to different pathological states [for review see 1, 3, 19, 23, 37].

Reactive oxygen species (ROS), i.e. the products of its incomplete reduction (Fig. 1), include: hydroxyl radicals ($^{\bullet}OH$), superoxide radical anion ($O_2^{\bullet-}$) and hydroperoxyl radical $HO_2^{\bullet}$ (a form with proton addition), and also the non-radical species: hydrogen peroxide (H_2O_2) and singlet oxygen $^1O_2$.

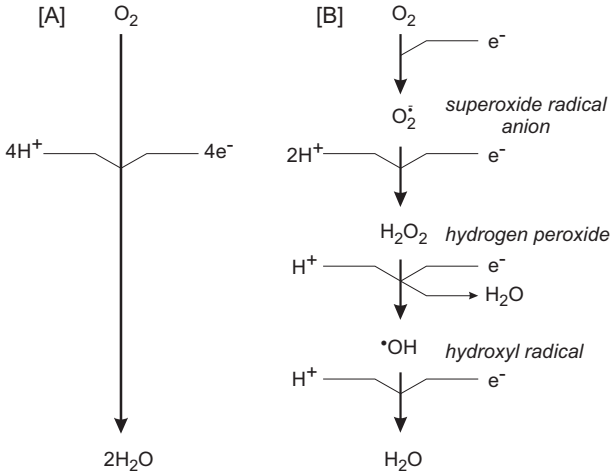


Fig. 1. Reduction of molecular oxygen to water (A) and successive one-electron reduction yielding reactive oxygen species ROS (B)

In the course of the most of biological reactions, $O_2^{\bullet-}$ is formed, which is transformed to H_2O_2 spontaneously or in enzymatic reaction catalyzed by superoxide dismutase (SOD) (reaction 1):

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^- \xrightarrow[\text{or (spontaneous)}]{\text{SOD}} O_2 + H_2O_2 \qquad (1)$$

H_2O_2 does not elicit direct strong oxidative action, but it can easily permeate across plasma membranes and oxidize transition metal ions (Fe^{+2}, Cu^{+1}), which results in the formation of reactive hydroxyl radical $^{\bullet}OH$ at different places within cell (reaction 2):

$$H_2O_2 + Fe^{+2} \longrightarrow {}^{\bullet}OH + OH^- + Fe^{+3} \qquad (2)$$

Superoxide radical anion can participate in repeated reduction of Fe^{+3} and regeneration of Fe^{+2} (reaction 3):

$$O_2^{\bullet-} + Fe^{+3} \longrightarrow O_2 + Fe^{+2} \qquad (3)$$

Combining the equations 2 and 3, we obtain reaction 4, called *biological Fenton reaction*:

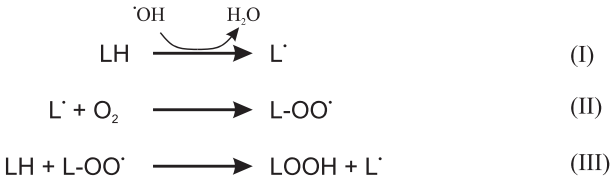
$$O_2^{\bullet-} + H_2O_2 \xrightarrow{Fe^{+2}/Fe^{+3}} {}^{\bullet}OH + OH^- + O_2 \qquad (4)$$

Therefore, in the presence of transition metals, appearance of one type of ROS in the cell creates a possibility of formation of remaining ROS.

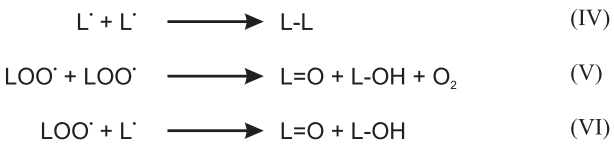
Oxidative stress in living cells

A characteristic feature of ROS is their exceptional reactivity caused by tendency to reach stability by pairing an electron. Thus, under certain circumstances electron(s) can be abstracted from some biologically important molecules, such as proteins, nucleic acids and lipids under the influence of free radicals. Lipids, the components of cell membranes, are particularly vulnerable to such attack. Electron abstraction from these molecules initiates free radical chain reaction, involving oxidation of fatty acids with the creation of peroxides, which is called lipid peroxidation [14, 40]. This process starts with H atom abstraction under the influence of $^{\bullet}OH$ radical to form alkyl radical $L^{\bullet}$ (reaction I). Lipid peroxide radicals ($L\text{-}OO^{\bullet}$) are formed next (reaction II), and they can abstract H atoms from other molecules with production of lipid peroxides (LOOH) (reaction III). $L\text{-}OO^{\bullet}$ can also react with lipid membrane and cytoplasmic proteins:

Cyclic, multiple repetition of reactions II and III corresponds to prolongation stage, which can be

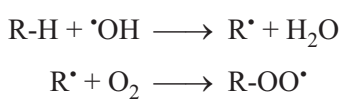


stopped by reactions between two free radicals with the formation of products, which are not free radicals any more, that is called termination stage (reactions IV, V, VI):



The enhanced peroxidation of membrane lipids leads to the introduction of polar peroxidic, carbonyl and hydroxyl groups into their molecules, which markedly diminishes their hydrophobicity and induces formation of toxic aldehydes and hydrocarbons.

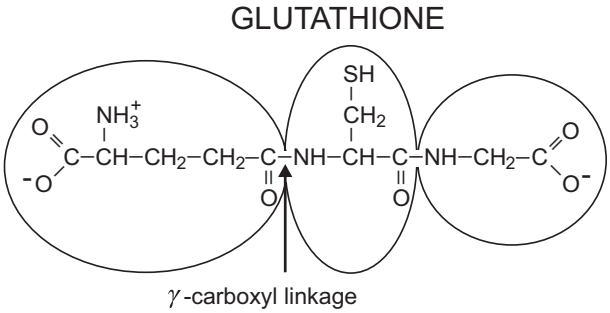
Not only lipids but also proteins [6, 7, 18, 36] and nucleic acids [38] can be subject to peroxidation, which, however, is not a cascade reaction. Protein molecules not so easily exchange unpaired electron, but rather they react with ascorbate or glutathione, thereby contributing to a decrease in cellular pool of low molecular weight redox buffers.



ROS are continuously generated during oxygen metabolism, but also momentarily neutralized by complex antioxidant system (so called ROS scavengers). Therefore, physiological level of ROS remains under very strict control. Danger appears only when a balance between pro- and antioxidants is disturbed, i.e. when detoxicant capabilities of the cells are outreached. Adverse ROS effects can be overcome by two independent mechanisms. The first one (and the most important) makes use of the enzymes, such as catalase, glutathione peroxidase and SOD, providing protection against Fenton reaction. The second mechanism is nonenzymatic and involves the action of reductive substances (antioxidants) in the cells, such as ascorbic acid, -tocopherol, -carotene, uric acid, and thiol compounds: glutathione and lipoic acid.

Thiols as biological antioxidant systems

Both, intracellular and extracellular redox states of thiols, play a critical role in the determination of protein structure and function, regulation of enzymatic activity, control of the activity of transcription factors and antioxidant protection [10, 35]. Antioxidant properties of thiol compounds depend on different mechanisms. These compounds can act as thiol/disulfide component of redox buffer, free radical scavengers and chelators of metal ions.



Biological action of cellular thiol compounds, such as glutathione (GSH), is related to the activity of hydrosulfide group which determines their ability to participate in antioxidant and detoxicant reactions [for review see 15, 25, 44].

As a thiol compound, glutathione fulfils a very important role of an antioxidant in the cells. GSH is relatively "resistant" to spontaneous oxidation, can enter a nonenzymatic reaction with hydroxyl radical (•OH) (cytotoxic product of Fenton reaction), and react with NO_3^-, $ONOO^-$, O_2 and $O_2^{\bullet-}$ [for review see 5, 10, 25].

Glutathione plays a significant role in detoxification of H_2O_2 and organic peroxides created during lipid peroxidation, in which glutathione peroxidase and glutathione reductase are engaged (Fig. 2). In antioxidant reactions, the reduced GSH is oxidized yielding disulfide (oxidized glutathione), that can be again reduced to thiol with the participation of glutathione reductase and NADPH. These reactions constitute an important redox cycle in the cells. When the peroxidative processes remain at physiological level, and at sufficient availability of NADPH and activity of glutathione reductase, high physiological GSH/GSSG ratio is maintained in normal range [10].

Antioxidant role of GSH consists also in its ability to react with organic free radicals (e.g. proteins and other molecules), which enables it to participate in regeneration of the damaged molecules.

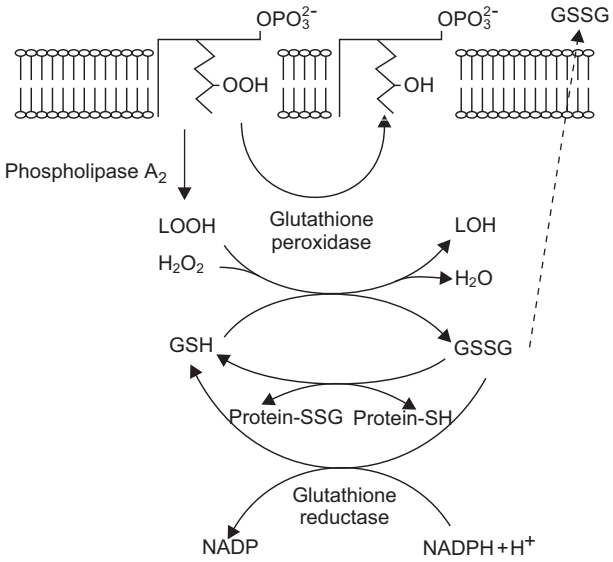
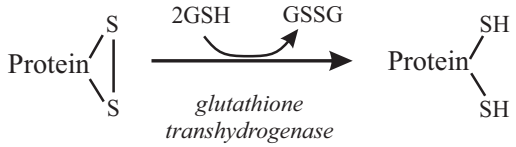


Fig. 2. Oxidation and reduction of glutathione by the enzymatic pathways

$$Protein^{\bullet} + GSH \longrightarrow Protein\text{-}H + GS^{\bullet}$$

In addition, –SH group plays an important antioxidant role by reducing disulfide bridges to free –SH groups. These reactions involving GSH can be nonenzymatic, but they are considerably accelerated by glutathione transhydrogenases (thiotransferases) [27].



H_2O_2 formed in the cells independently of glutathione peroxidase activity can also be reduced by catalases present in peroxisomes (Fig. 3), while glutathione peroxidase function is particularly important in mitochondria, where catalases are absent [12]. Therefore, mitochondrial GSH plays a critical role in protection against ROS formed in this structure.

Strong oxidative and nitrosylative stress quickly and efficiently diminishes GSH level in the cells. Hence, aggravation of peroxidative injury and NADPH deficit (due to decreased activity of glucose-6-phosphate dehydrogenase) leads to the accumulation of glutathione disulfide (GSSG). Oxidized glutathione (GSSG) is not only a useless metabolite but it is also highly dangerous to the cells. The increased

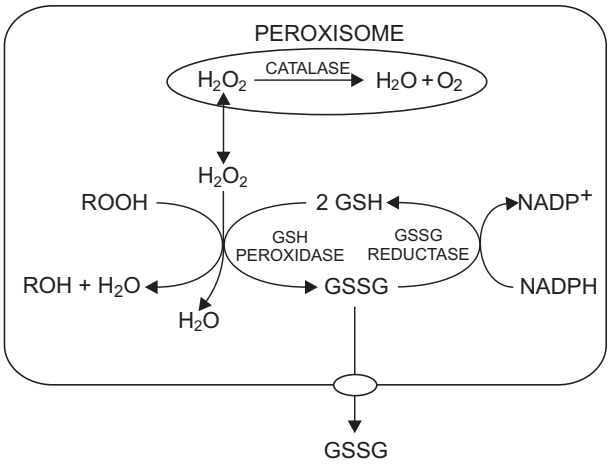


Fig. 3. Hydrogen peroxide biodegradation by GSH peroxidase in the cytosol and by catalase in peroxisome

GSSG level facilitates formation of mixed disulfides, i.e. protein glutathionylation, which induces changes in redox status of thiols [22]. Consequently, it affects so important biological processes in the cells as regulation of gene transcription, activity of enzymes and receptors. Another mechanism protecting the cells against GSSG excess involves its translocation outside the cells which, however, leads to a drop in intracellular glutathione pool [25].

Formation of dangerous thiyl radicals (–S$^{\bullet}$)

Biothiols are extraordinarily efficient antioxidants protecting the cells against consequences of damage induced by free radicals due to their ability to react with the latter. In antioxidant reactions, thiols undergo one-electron oxidation with the formation of thiyl radicals [43]

$$RSH \longrightarrow RS^{\bullet} + H^+ + e$$

Thiyl radicals are produced in all reactions of thiols with other free radicals and with H_2O_2:

$$RSH + R'^{\bullet} \rightarrow R'H + RS^{\bullet}$$

$$RSH + O_2^{\bullet-} + H^+ \longrightarrow RS^{\bullet} + H_2O_2$$

$$2RSH + H_2O_2 \longrightarrow 2RS^{\bullet} + 2H_2O$$

$$RSH + {}^{\bullet}OH \longrightarrow RS^{\bullet} + H_2O$$

Thiyl radicals (RS$^{\bullet}$) are formed also in photolysis of disulfides:

$$RSSR \xrightarrow{h} 2RS^{\bullet}$$

Furthermore, they can be generated in the reactions of thiols with transition metal ions, characterized by bearing an unpaired electrons in their inner electronic shell:

$$RSH + Me^{n} \longrightarrow RS^{\bullet} + Me^{n-1} + (H^{+})$$

Transition metal ions, capable of changing their oxidation state, become very often promoters of free radical reactions, such as biological Fenton reaction [2]. These reactions involve not only iron ions, but also ions of other transition metals present in the cells or entering the organism from the polluted environment.

$$O_2^{\bullet-} + H_2O_2 \xrightarrow{Fe^{+2}/Fe^{+3}} {}^{\bullet}OH + OH^{-} + O_2$$

Some biothiols, such as cysteine regarded a very toxic amino acid, can cause lipid peroxidation by reducing metal ions (in biological systems mainly iron and copper ions) [2, 20]. Thiol compounds can exert their prooxidative action by the reduction of Fe^{+3} to Fe^{+2}, leading both, to the formation of thiyl radical ($RS^{\bullet}$) and to excessive generation of superoxide radical anion ($O_2^{\bullet-}$).

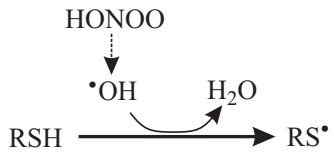
$$RS^{-} + Fe^{+3} \longrightarrow RS^{\bullet} + Fe^{+2}$$

$$Fe^{+2} + O_2 \longrightarrow Fe^{+3} + O_2^{\bullet-}$$

It indicates that thiol compounds in the presence of trace amounts of transition metal ions and oxygen are oxidized to form thiyl radicals and $O_2^{\bullet-}$, and, in consequence, also other reactive oxygen species. For individual thiol compounds, this danger is the higher the lower pK_a value of hydrosulfide (–SH) group is, since thiolate ion reduces metal ions much quicker than undissociated –SH group does.

Thiyl radicals are also formed in the reaction of thiol compounds with highly oxidative peroxynitrite ($ONOO^{-}$) [4, 26]. Homolytic cleavage of peroxynitrous acid (HONOO) leads to the release of hydroxyl radical (${}^{\bullet}OH$) capable of oxidation of thiol compounds to form thiyl radicals. This reaction is considered the main cause of peroxidative damage provoked by $ONOO^{-}$ in the cells [4, 21, 31]:

$$ONOO^{-} + H^{+} \qquad HONOO \qquad HO^{\bullet} + NO_2^{\bullet}$$



Alternatively, thiol compounds can be oxidized in two-electron process with the formation of unstable sulfenic acids, whose immediate reaction with thiol compounds yields disulfides [8]:

$$RSH + ONOO^{-} \longrightarrow RSOH + NO_2^{-}$$

$$RSOH + R'SH \longrightarrow RSSR' + H_2O$$

Mechanisms of chemical reactions involving thiyl radicals

Thiol compounds attract interest almost exclusively for their antioxidant properties. Considerably less attention is paid to thiyl radicals ($RS^{\bullet}$) formed simultaneously in these reactions. However, protective and repairing efficacy of thiols depends not only on their ability to detoxify free radicals but also on chemical character and reactivity of the formed thiyl radical ($RS^{\bullet}$) [32, 41, 43]. Antioxidant action of thiol compounds is determined by both, efficient detoxification of free radicals and inactivation of the concurrently created thiyl radical. Furthermore, quick and efficient removal of $RS^{\bullet}$ radical leads to a disturbance in balanced state of antioxidant reaction, which effectively increases repairing capacity [32, 43]. For this reason, thiol compounds, similarly as any compounds able to play a role of antioxidants, should immediately undergo regenerative reduction:

$$RS^{\bullet} + e^{-} \longrightarrow RS^{-}$$

$$RS^{\bullet} + e^{-} + H^{+} \longrightarrow RSH$$

The values of standard redox potentials of both these half-reactions (E_0 = 0.75 V, E_0 = 1.33 V) clearly indicate that, under physiological conditions, thiyl radicals are relatively strong oxidants [32, 33]:

$RS^{\bullet} + D^{-}$ $RS^{-} + D^{\bullet}$ D^{-} = electron donor

$RS^{\bullet} + DH$ $RSH + D^{\bullet}$ DH = hydrogen donor

Characteristic feature of thiyl radicals is their ability to abstract hydrogen atoms from other molecules. Thiyl radicals ($RS^{\bullet}$) can also participate in electron transfer reaction and intramolecular rearrangements of free radicals.

The reaction of thiyl radicals with thiolate anion leads to the formation of disulfide radical anions, and the rate of this reaction is determined by dissociation constant of –SH group [42, 43, 46]:

$$RSH \qquad RS^- + H^+$$

$$RS^\bullet + RS^- \longrightarrow (RSSR)^{\bullet-}$$

disulfide radical anion

The reaction of disulfide radical anion with molecular oxygen yields disulfide (RSSR) and superoxide radical anion ($O_2^{\bullet-}$), one of ROS [46]:

$$(RSSR)^{\bullet-} + O_2 \longrightarrow RSSR + O_2^{\bullet-}$$

Addition reactions of thiyl radicals with oxygen produce dangerous peroxyl radicals, which can generate further free radicals in subsequent reactions with thiol compounds [32, 33]:

$$RS^\bullet + O_2 \qquad RSOO^\bullet$$

$$RSOO^\bullet + RSH \longrightarrow RSO^\bullet + RSOH$$

$$RSOO^\bullet + RSH \longrightarrow RSOOH + RS^\bullet$$

Therefore, the reactions of thiyl radical with thiolate anion (RS^-) are considered less dangerous in comparison with the reaction of thiyl radical with oxygen to form thiyl peroxyl radicals ($RSOO^\bullet$). In the latter case, chain reactions with free radical formation can propagate, while $O_2^{\bullet-}$ (produced in the reaction of $RSSR^\bullet$ with O_2) can be inactivated by SOD:

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Glutathione (GSH) is a thiol tripeptide ubiquitous in the cells and occurring at high concentrations. Reactions of glutathione (GSH) with free radicals yield thiyl radical ($GS^\bullet$), whose subsequent reactions lead to GSSG *via* ($GSSG^{\bullet-}$), and $O_2^{\bullet-}$ inactivated by SOD [45, 46]. Therefore, close cooperation between GSH and SOD is necessary for antioxidant action of GSH, the main cellular thiol compound [45, 46]. That is why Winterbourn [45, 46] put forward the hypothesis that tight cooperation between GSH and SOD is an important determinant of efficient removal of free radicals generated in the cells.

H_2O_2 and organic peroxides (R-OOH) are reduced by glutathione peroxidase containing selenocysteine in its active center, which enables two-electron oxidation of GSH yielding glutathione disulfide (GSSG) without the formation of glutathione thiyl radical ($GS^\bullet$) (Fig. 4) [13]. Glutathione peroxidase can also oxidize thiol groups in proteins to form disulfides. Therefore, the question arises what physiological role this enzyme plays. Is this reduction of hydroperoxides or oxidation of thiol compounds [24]?

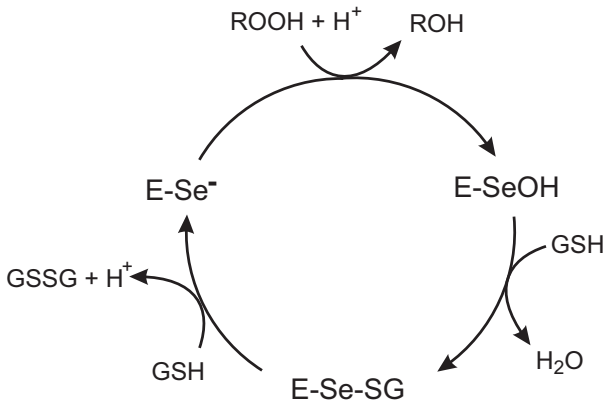


Fig. 4. Mechanism of catalytic action of glutathione peroxidase [13]

Thiyl radicals can react with unsaturated fatty acids, being directly added to double bonds [11]:

$$R_1\text{–CH=CH-CH=CH–}R_2 + RS^\bullet \rightarrow R_1\text{–CH(RS)-}^\bullet\text{CH-CH=CH–}R_2$$

(free radical at carbon atom)

This process is accompanied by *cis*/*trans* isomerization which causes an increase in a number of *trans* double bonds in fatty acids [39]. Such isomerization is characteristic of the reactions of unsaturated fatty acids with thiyl radical. None of other free radicals: alkyl ($R^\bullet$) or $^\bullet OH$ exhibits ability to induce such stereochemical transformation. In this way, lipid structure is changed as a result of reaction of thiyl radicals with membrane unsaturated fatty acids, which alters packing and density of bilayer lipid cell membrane and disturbs its biological function [16, 34].

Due to its capacity to pair electron, thiyl radical can also abstract hydrogen from lipids, thereby initiating peroxidative lipid damage:

$$LH \xrightarrow{R\text{-}S^\bullet \quad RSH} L^\bullet$$

Thiyl radicals can undergo dimerization to yield disulfides which terminate the reaction:

$$RS^\bullet + RS^\bullet \qquad RSSR$$

Thiyl radicals can also participate in abstraction of hydrogen atom from organic compounds, for instance a radical centered at secondary carbon atom is generated from alcohol [33, 43]:

$$RS^{\bullet} + (CH_3)_2CHOH \qquad RSH + (CH_3)_2{}^{\bullet}COH$$

Moreover, in one-electron reactions thiyl radicals can oxidize substrates, that are electron donors [47]:

$$RS^{\bullet} + \text{reductant} \qquad RS^{-} + \text{radical}^{\bullet}$$

Ascorbate (AH^-) oxidation yielding ascorbyl radical can be an example of the reaction with electron transfer in which thiyl radicals participate [42, 43].

$$RS^{\bullet} + AH^{-} \qquad RSH + A^{\bullet-}$$

Another example of single electron transfer (not constituting a part of thiyl radical but closely connected with it) is provided by the above-mentioned reaction of disulfide radical anion $(RSSR)^{\bullet-}$ with oxygen:

$$(RSSR)^{\bullet-} + O_2 \longrightarrow RSSR + O_2^{\bullet-}$$

With regard to glutathione thiyl radical, intramolecular, tautomeric rearrangement of hydrogen atom was observed, resulting in the possibility of formation of radicals centered at one of carbon atoms [17]:

$$\begin{array}{l} {}^{+}H_3N\text{—}\,CH.....CH_2S^{\bullet} \\ \qquad\quad | \\ \qquad\quad COO^{-} \end{array} \leftrightarrow \begin{array}{l} {}^{+}H_3N\text{—}\,{}^{\bullet}C.....CH_2SH \\ \qquad\quad | \\ \qquad\quad COO^{-} \end{array}$$

Tautomerization of mercaptoethanol thiyl radical can also lead to the generation of a radical with unpaired electron at carbon atom [47]:

$$HOCH_2CH_2S^{\bullet} \qquad HOCH_2{}^{\bullet}CHSH$$

Antioxidant reactions of thiols yield thiyl radicals ($RS^{\bullet}$) first, then disulfide radical anions $(RSSR)^{\bullet-}$ and finally disulfides and $O_2^{\bullet-}$, which is next inactivated in the reaction catalyzed by SOD [47].

Another antioxidant in the cells, vitamin C, plays also very important role in detoxification of thiyl radicals. Under physiological conditions, the reaction of glutathione thiyl radical ($RS^{\bullet}$) with ascorbate can be much quicker than its reaction with thiolate anion or oxygen. Standard redox potential of one-electron reduction of ascorbate amounts to $E_0(AH^{\bullet}/AH^{-}) = 0.28$ V while $E_0(GS^{\bullet}/GSH) = +0.9$ V, so glutathione thiyl radicals can oxidize ascorbate [43]:

$$GS^{\bullet} + AH^{-} \qquad GSH + A^{\bullet-}$$

Ascorbyl radical created in this reaction is less reactive than glutathione thiyl radical, which makes ascorbate an excellent antioxidant. The formed ascorbyl radicals can subsequently enter disproportiation reaction:

$$2AH^{\bullet} \qquad \text{ascorbate} + \text{dehydroascorbate}$$

Therefore, comparison of two the most important antioxidant compounds in the cells, ascorbate and GSH shows that GSH, unlike ascorbate, is a potential source of such radicals as $GS^{\bullet}$, $(GSSG)^{\bullet-}$, $GSSG^{\bullet}$ and $O_2^{\bullet-}$ and requires SOD cooperation.

Thiyl radicals are efficiently detoxicated by reaction with ascorbate, which simultaneously increases antioxidant and repairing capacity of thiol compounds. This is also an example of cooperation between the most important antioxidants in the cells, i.e. thiols and ascorbate. There is a question whether $O_2^{\bullet-}$ radical (decomposed by SOD) or ascorbyl radical is the final product of free radical detoxification. Wardman [43] believes that reaction of thiyl radicals ($RS^{\bullet}$) with ascorbate with the formation of ascorbyl radical is the most probable mechanism associated with "repairing" action of thiols.

Important biological function of thiols consists in their participation in oxidation and reduction reactions, in which sulfur of thiol groups passes to higher oxidation state, as we can observe with aerobic biodegradation of cysteine. The main products of oxidation of hydrosulfide sulfur (–SH) include: thiyl radicals, sulfenic acids, sulfinic acids, sulfonic acids and corresponding radicals [9]. Oxidation of thiol compounds to sulfinic and sulfonic acids is an irreversible process which usually leads to the loss of biological activity.

Humans and animals are continuously exposed in their environment to numerous exogenous thiol compounds and related disulfides. Thiol compounds are present in food, environmental pollutants or are produced during biodegradation of sulfur-containing compounds.

Aromatic thiol compounds, benzenethiol derivatives occur in roasted meat while furano-3-thiol has been found in fish meat [28, 29]. Various food

products and condiments are supplemented with thiol compounds to improve their flavor and aroma. Certain compounds occurring in plants can be toxic to some animals for ability of their transformation into thiol compounds. For instance, vegetables related to onion and summer squash contain cysteine alkylsulfoxides, whose biodegradation yields methanethiol [30]. These plants are toxic to sheep and cattle which fall ill with severe anemia just due to the formation of methanethiol and corresponding dimethyldisulfide. Propenethiol present in onion is toxic to dogs and cats due to its hemolysis-evoking ability [28].

Hemolysis, which can be provoked in animals by plant thiol compounds, is initiated by one-electron oxidation reaction of thiolate anion (RS^-) to form dangerous thiyl radical ($RS^\bullet$) in which hemoglobin participates. Consequently, excessive formation of H_2O_2 and other ROS in erythrocytes leads to the disturbances in pro- and antioxidant balance, peroxidation of membrane lipids and hemolysis [29]:

$$HbFe^{II}O_2 + RS^- + 2H^+ \longrightarrow HbFe^{III} + RS^\bullet + H_2O_2$$

There have been no reports on toxic action of plant thiols in humans. Hemolysis and anemia caused by exogenous thiol compounds and other xenobiotics were observed only in the patients suffering from deficits of glucose-6-phosphate dehydrogenase activity in erythrocytes, which was associated with deficit of glutathione, one of the main cellular antioxidants.

In conclusion, reductive ability of thiol compounds towards free radicals is associated with the formation of thiyl radical ($RS^\bullet$), and the rate and efficiency of its removal have critical effect on antioxidative or prooxidative actions of thiols in the cells. Current studies into formation and reactivity of thiyl radicals focus mainly on low molecular weight compounds. In the future, it seems necessary to extend these studies to high molecular weight thiol compounds, namely proteins and respective protein thiyl radicals ($P\text{-}S^\bullet$).

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