The Chemistry of Antioxidants*

2. Metal-free Enzymatic Antioxidants

K. Hussain Reddy

The chemistry of metalloenzymatic antioxidants viz. superoxide dismutase (SOD), catalase (CAT), peroxidases was discussed in the first article. This second part illustrates the structural and mechanistic aspects and antioxidant properties of metal-free antioxidant enzymes viz. thioredoxin, peroxiredoxin, glutathione S-transferase, and glutathione peroxidase.

1. Thioredoxin

Thioredoxin (Trx) is a ubiquitous small protein. Though it occurs in many types of cells, it was first identified in *E. coli*. An essential enzyme in mammals, Trx is known to actively participate in protecting cells against reactive oxygen species (ROS). Trx plays a central role in humans and are increasingly linked to medicine through their response to ROS. In humans, Trx is encoded by two genes—*TXN* and *TXN2*. Mutation in either of these genes is lethal for developing embryos. In plants, Trx is known to regulate a spectrum of fundamental processes such as photosynthesis, growth, flowering, communication, and seed germination.

*Structure*

Thioredoxin is a monomer. The 12 kD protein has 108 amino acids with a pair of neighbouring cysteine residues. The enzyme contains redox-active dithiol motifs in the active site that possess a highly conserved -Trp-Cys-Gly-Pro-Cys-Lys- sequence. The X-ray structure of Trx reveals that the enzyme’s active disulphide

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**Keywords**

Thioredoxin, peroxiredoxins, glutathione S-transferases, glutathione peroxidase, structure, function, mechanism of action, enzymes, antioxidants.
The X-ray structure of thioredoxin reveals that the enzyme’s active disulphide group is located on a molecular protrusion, making this protein the only known example of a ‘male’ enzyme. The schematic structure of Trx is shown in Figure 1.

Trx, isolated from *E. Coli*, is one of the best-characterized proteins. Though Trx has been isolated from different sources, the enzyme has the same overall 3D structure, and the number of amino acid residues is highly conserved: Asp-26, Ala-29, Trp-31, Asp-61, Pro-76, and Gly-92. Solution NMR studies indicate that the overall changes between reduced and oxidized forms are minimum. However, studies suggested that the structure has a local conformational change near the redox-active site. The cysteine residue in the N-terminal active site has a low pKa value. Hence it has been regarded as the attacking nucleophile in disulfide reduction. The enzymatic reaction is reversible. Depending on the redox potential of the substrate, the enzyme may either break or form disulfides. In plants, the proteins form a novel type of cellular communication system.
Functions

Thioredoxins are known to act as antioxidants by facilitating the reduction of other proteins by cysteine thiol-disulfide exchange. Important biochemical functions of Trx are summarized in Table 1. These enzymes play a vital role in redox signalling. Trx reductase protects the enzyme system from oxidizing agents. Reduced Trx activates both fructose-1,6-bisphosphatase (FBPase) and sedoheptulose-1,7-bis-phosphatase (SBPase) by a disulphide interchange reaction. Trx reacts with inactive bisphosphatases by disulphide interchange, thereby activating these flux controlling enzymes of the Calvin cycle. Trx is known to reduce oxidative stress and inflammation and increase longevity by 35% in laboratory animals. These proteins take part in protein methylation reactions.

Trx not only acts as an electron donor but also works as a catalyst in the reduction of ribonucleotides. It is involved in the conversion of ribonucleotides to deoxyribonucleotides and supplies reducing equivalents. NADPH-dependant thioredoxin reductase converts the oxidized thioredoxin to the reduced form, which can be recycled. Thus Trx serves as a protein co-factor in an enzymatic reaction.

Trx plays a key role in coordinating light and dark reactions of photosynthesis. Trx is reduced in the presence of light abundantly. In the reduced form, it activates many biosynthetic systems. Thus the activity of Trx depends on the reduction of its disulphide bridge (See Figure 1). The catalytic activity of phosphoribulokinase, for example, increases by 100-fold on illumination. Conversely, several degradative enzymes are inhibited when their disulphide bridges are reduced by Trx. In chloroplasts, oxidized Trx is reduced by ferredoxin in a reaction catalyzed by ferredoxin-thioredoxin reductase. Thus, the activities of the light and dark reactions of photosynthesis are coordinated through the reduction potential of ferredoxin and thioredoxin.
<table>
<thead>
<tr>
<th>Biochemical Function</th>
<th>Organism</th>
<th>Comments and References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduction of $H_2O_2$</td>
<td>Many</td>
<td>Many peroxiredoxin, catalyzing the reduction of $H_2O_2$ and thereby preventing oxidative stress and apoptosis induction, require Trx.</td>
</tr>
<tr>
<td>Protein disulphide reduc-</td>
<td>All</td>
<td>Trx plays a key role in the reduction of intracellular protein disulphides.</td>
</tr>
<tr>
<td>tion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein repair</td>
<td>Many</td>
<td>Trx is hydrogen donor for methionine sulphoxide reductase.</td>
</tr>
<tr>
<td>DNA synthesis</td>
<td>All</td>
<td>Trx is a hydrogen donor for ribonucleotide reductase.</td>
</tr>
<tr>
<td>Regulation of photosyn-</td>
<td>Plants</td>
<td>Photosynthesis regulation by light via ferredoxin.</td>
</tr>
<tr>
<td>thesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regulation of transcription</td>
<td>Mammals</td>
<td>Different transcription factors are either activated or inhibited by Trx.</td>
</tr>
<tr>
<td>Regulation of apoptosis</td>
<td>Mammals</td>
<td>Trx-(SH)$_2$ makes a complex with apoptosis signal-regulating kinase 1 (ASK1) preventing downstream signalling for apoptosis.</td>
</tr>
<tr>
<td>Immunomodulation</td>
<td>Mammals</td>
<td>Extracellular Trx is involved.</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Mammals</td>
<td>Trx from cytotrophoblasts assist implantation and establishment of pregnancy.</td>
</tr>
<tr>
<td>Birth</td>
<td>Mammals</td>
<td>Protection from hyperoxia at birth by induction of Trx.</td>
</tr>
</tbody>
</table>

**Table 1.** Biochemical functions of thioredoxin (Trx) in different organisms.

**Mechanism of Action**

The enzyme system primarily reduces oxidized cysteine residues and cleaves disulfide bonds. Thioredoxin reductase reduces the oxidized Trx, and the former, in turn, is reduced by NADPH. The cysteine moieties can be oxidized to the corresponding disul-
fide that is reduced, in turn, by thioredoxin reductase (TR), an NADPH-dependent selenoflavoprotein (Figure 2).

Though Trx exists in several forms, cytosolic (Trx-1) and mitochondrial (Trx-2) are most widespread. Their functions are supportive but not overlapping. They act as a major antioxidant in the heart. Trx regulates micro RNA levels in the heart and has been found to inhibit cardiac hypertrophy. It is known to mediate the inhibition of ventricular remodelling. It is thought to indirectly modulate protein methylation for cardiac protection. It also interacts with important signalling molecules and transcription factors for regulating various cellular functions. Since the enzyme acts as an antioxidant, it has been used in skincare products in association with glutaredoxin and glutathione.

Trx plays a crucial role in the light activation mechanism (Figure 3) of biphosphatases (FBPase and SBPase) in chloroplasts. Photoactivated PSI reduces soluble ferredoxin (Fd), which, in turn, reduces the disulphide linkage of Trx. Fd_{red} reacts with the inactive biphosphatases in disulphide interchange, thereby activating the Calvin cycle. The terminal step of the ribonucleotide reductase catalytic cycle is the reduction of the enzyme’s newly formed disulphide bond to re-form its redox active sulphhydryl pair.

Trx is one of the physiological reducing agents (Figure 4). It reduces oxidized ribonucleotide reductase via disulphide interchange.
Figure 3. Light activation mechanism of bisphosphatases.

Figure 4. Re-formation of redox active sulphhydryl pair of ribonucleotide reductase.

2. Peroxiredoxins

Peroxiredoxins (Prxs) are highly conserved proteins that have been identified in all phyla. These enzymes make up an omnipresent family of thiol-dependent peroxidases. The functionally conserved enzyme systems have been isolated from yeast, bacteria, and mammals, indicating the importance of a defence system for protecting against ROS during the evolution of living organisms. The relative abundance of these proteins reflects the physiological importance of these enzymes. Recent reports on the reactivity of Prxs revealed that these are also prominent members of the antioxidant defence network. Many species contain more than one type of peroxiredoxin (Prx). For example, mammals have six different Prxs, with Prxs 1, 2 and 6 located in the cytosol, Prx3 in the mitochondrial matrix, Prx4 in the endoplasmic reticulum, and Prx5 in the mitochondria, peroxisomes and cytosol.

Peroxiredoxins are often referred to as alkyl hydroperoxide reductase (AHPC) in bacteria. These are also called thiol specific antioxidants (TSA) and thioredoxin peroxidase (TPx). Prx2 is one of the most abundant proteins in red blood cells after haemoglobin.
Deficiency of peroxiredoxins causes haemolytic anaemia, hyperoxia and certain types of haematopoietic cancers.

Structure

Peroxiredoxins (Prxs) may be classified into three types namely—typical 2-Cys Prxs, atypical 2-Cys Prxs, and 1-Cys Prxs. Crystal structures of six Prxs have been reported. These structures indicate that all Prxs are similar, each containing a Trx fold.

The enzyme systems have different oligomeric states: Atypical 2-Cys Prxs are monomeric, while the typical 2-Cys and the 1-Cys Prxs are homodimers. The active site is highly conserved among the Prx classes. It was observed that among 22 Prx sequences, only one cysteine residue (the prime active site) was absolutely conserved. At present, it is considered as the required peroxidatic cysteine, C_P. The second semi conserved cysteine is the resolving cysteine, C_R, which forms an inter-unit disulphide bond with C_P. Eventually, it was known that C_R can occupy several positions in various Prx families. Unlike the heme-containing catalases and selenium dependant glutathione peroxidases, these enzymes do not require cofactors. C_R is present in five locations of the structure, giving either an inter-subunit or intra-subunit disulphide bond in oxidized protein depending on the position of C_R.

Functions

Peroxiredoxins (Prxs) are a class of thiol peroxidases that degrade hydroperoxides to water. It is one of the most important enzyme systems, together with superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), which defends cells against oxidative stress. This family of proteins catalyze the reduction of hydrogen peroxide (H_2O_2), alkyl hydroperoxides and peroxynitrite to water, the corresponding alcohol and nitrite, respectively. These proteins emerged as scavenging enzymes for highly toxic substances such as peroxide and peroxynitrite in biological systems. The role of peroxiredoxins was overshadowed for a long time by well-studied oxidative stress defence en-
**Figure 5.** Mechanism of action of three Prx subtypes

In typical 2-Cys Prxs, the main cysteine residue (C$_P$) reacts with the residue C$_R$ on the second subunit of the dimer. In atypical 2-Cys Prxs, the oxidized C$_P$ reacts with C$_R$ residue located in the same molecule. In 1-Cys Prxs, the C$_P$ residue generates sulfenic acid and is regenerated directly through donation of an electron to the thiol form in the presence of ascorbate. C$_P$, cyclophilin; Grx, glutaredoxin; GSH, reduced glutathione; ROOH, peroxide; C$_P$, peroxidatic Cys; C$_R$, resolving cysteine; Trx, thioredoxin.

Enzymes such as catalase and glutathione peroxidase, which were considered the major enzymes responsible for protecting cells against hydroperoxides. These enzymes play a role in cell signalling by modulating H$_2$O$_2$ levels. In plants, these enzymes protect the chloroplasts against photo-oxidative damage.

**Mechanism of Action**

*Figure 5* shows the mechanism of action of the three PRDX subtypes. C$_P$ is oxidized to sulfenic acid (H$_2$SO$_3$) by the peroxide substrate. Recycling of H$_2$SO$_3$ back to thiol may be used to distinguish the three enzyme types. 1-Cys enzymes may be reduced by vitamin C or reduced glutathione (GSH), while 2-Cys enzymes are reduced by thiols of thioredoxins and glutathione. The enzyme becomes inactive due to hyperoxidation of active...
thiol to sulfinic acid. The reaction can be reversed by sulfiredoxin. Peroxiredoxins are modulated by phosphorylation. The redox status of the enzyme is maintained by some chemical reactions and oligomerization states. After reducing H₂O₂, peroxiredoxin makes use of Trx to restore to its original state, as shown below.

\[
\text{Peroxiredoxin (red)} + \text{H₂O₂} \rightarrow \text{Peroxiredoxin (ox)} + 2\text{H₂O} \quad (1)
\]

\[
\text{Peroxiredoxin (ox)} + \text{Thioredoxin (red)} \rightarrow \text{Peroxiredoxin (red)} + \text{Thioredoxin (ox)} \quad (2)
\]

**Applications**

These enzymes are highly sensitive to oxidation by peroxides. Hence Prxs serve as sensors and transducers of H₂O₂ signalling. The role of Prx enzymes is not limited to their peroxidase activity but also other related functions such as regulation of signalling and metabolism, oxidation of redox-sensitive signalling proteins, and protein folding. The disappearance of its antioxidant function in the individual gene knockouts of Prx1 and Prx5 has been shown to induce tumorigenesis.

1Chemical substances foreign to the body or to an ecological system.

3. **Glutathione S-transferases (GSTs)**

Glutathione S-transferases (GSTs) belong to a family of eukaryotic and prokaryotic phase II metabolic isozymes. These are detoxifying enzymes, and catalyze the conjugation of the reduced form of glutathione (GSH) to a xenobiotic substrate for detoxification.

**Structure**

GSTs were known as ligandins. The enzyme system comprises (i) cytosolic, (ii) mitochondrial, and (iii) microsomal proteins. These GSTs are detoxifying enzymes, and catalyze the conjugation of the reduced form of glutathione (GSH) to a xenobiotic substrate for detoxification.
proteins have different amino acid sequences. In mammals, the enzymes constitute up to 10% of cytosolic proteins. Cytosolic GSTs are divided into 13 classes based on their structure—\textit{alpha, beta, delta, epsilon, zeta, theta, mu, nu, pi, sigma, tau, phi, and omega}. Mitochondrial GSTs belong to the class kappa. Microsomal GSTs comprise four subgroups designated with roman letters I, II, III, and IV in which amino acid sequences share less than 20% identity. Human cytosolic GSTs belong to the \textit{alpha, zeta, theta, mu, pi, sigma, and omega} classes. GSTs are globular proteins. The structure of the porcine \textit{pi-class} enzyme (pGTSP1-1) was the first to be determined. It has a thioredoxin-like N-terminal domain and a C-terminal domain consisting of alpha helices. Mammalian cytosolic GSTs are dimeric. The monomer is approximately 25 kDa in size.

\textbf{Functions}

GSTs catalyse the conjugation of glutathione (GSH) via a sulfhydryl group to electrophilic centres on a wide range of substrates to make the compounds more water-soluble. This activity detoxifies endogenous compounds such as peroxidised lipids and enables the breakdown of xenobiotics. GSTs may also bind toxins and function as transport proteins, an activity that gave rise to its early name—'ligandin'.

The main function of GSTs is to detoxify xenobiotics. The enzyme catalyzes the nucleophilic attack by GSH on electrophilic carbon, sulphur, or nitrogen atoms of non-polar xenobiotic substrates. As a result, GSTs prevent the reaction of xenobiotics with DNA, RNA, and cellular proteins. GSTs bind both the substrate and GSH to form an active site that activates the thiol group of GSH, facilitating the nucleophilic attack upon the substrate. GSTs target exogenous toxins (Figure 6) such as pesticides, herbicides, epoxides, carcinogens, drugs, chemotherapeutic agents, etc.

Biochemical functions of these proteins are summarized in Table 2.
Applications

In plants, GSTs provide tolerance toward herbicides used in agriculture. They are also known to involve in hormone biosynthesis, tyrosine degradation, peroxide breakdown, stress signalling by proteins, nodule function, and non-catalytically acting as flavonoid-binding proteins. GSTs are also involved in the modulation of cell signalling kinases, formation and modulation of ion channels, redox reactions, and the glutathionylation of proteins.

4. Glutathione Peroxidase

Glutathione (GSH) is present in high levels in animals, plants, and bacteria. It acts as a redox sulfhydryl buffer. It cycles between the

**Figure 6.** The compounds targeted by GST

A) Detoxification pathway of 4-hydroxynonenal involving GST; B) GST-catalyzed conjugation of acrolein and subsequent transformation steps C) enzymatic inactivation of cisplatin catalyzed by GSTs; D) first step of the metabolic pathway of busulfan; E) enzymatic inactivation of dichoroacetate leading to glyoxylate.
<table>
<thead>
<tr>
<th>Toxic compound</th>
<th>Source</th>
<th>Toxicity</th>
<th>Function of enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin</td>
<td>Aspergillus</td>
<td>Highly toxic to liver, risk factor for hepatocellular carcinoma</td>
<td>Catalyzes the conjugation of AFB1-exo-8,9-epoxide to endogenous GSH.</td>
</tr>
<tr>
<td>Aflatoxin B₁</td>
<td>Cereals, groundnuts</td>
<td>Binds guanine residues of DNA and confer mutagenic properties</td>
<td>Same as above.</td>
</tr>
<tr>
<td>4-Hydroxynonenal (4-HNE)</td>
<td>Formed due to lipoperoxidation</td>
<td>Damage proteins and DNA</td>
<td>Catalyzes the conjugation of 4HNE to GSH.</td>
</tr>
<tr>
<td>Acrolein</td>
<td>Chemical industries, lipid oxidation, as biocide in agriculture, burning of tobacco, plastic, oils, etc.</td>
<td>Highly toxic, carcinogenic</td>
<td>Catalyzes conjugation of acrolein to GSH.</td>
</tr>
</tbody>
</table>

Table 2. Biochemical functions of GSTs in different organisms.

Reduced thiol form (GSH) and oxidized disulphide form (GS-SG), in which the tripeptides are linked by a disulphide bond. The GS-SG is reduced to GSH by glutathione reductase, a flavoprotein that uses NADPH as the electron source. The ratio of GSH to GS-SG is more than 500 in most cells.

Glutathione plays a key role in detoxification by reacting with H₂O₂ and organic peroxides, the harmful byproducts of aerobic life.

\[
2\text{GSH} + \text{R-O-OH} \rightarrow \text{GS-SG} + \text{H}_2\text{O} + \text{ROH} \quad (3)
\]

Reduced GSH is essential for maintaining the normal structure of red blood cells and keeping haemoglobin in the ferrous state. Cells with a lower level of reduced GSH are more susceptible to hemolysis.
It is a tripeptide (Figure 7) consisting of glycine, glutamate and cysteine amino acid residues. It protects the -SH groups of proteins in the reduced state.

It serves as a reducing agent for glutaredoxin in deoxyribonucleotide synthesis. An important function of GSH in red blood cells is to reductively eliminate reactive oxygen metabolites, viz. hydrogen peroxide and organic hydroperoxides. These ROS can reversibly damage Hb and break C-C bonds in the phospholipid tails of the cell membrane. The uncontrolled accumulation of peroxides causes premature cell lysis, i.e., break down of cell membrane.

Peroxides are scavenged by a reaction with glutathione, a reaction catalyzed by glutathione peroxidase. Reduced glutathione (GSH) is subsequently regenerated by the reduction of GS-SG by NADPH. This reaction is catalyzed by glutathione reductase. The presence of NADPH is therefore vital for the uprightness of erythrocytes.

Occurrences and functions of isozymes of glutathione peroxides encoded by different genes are given in Table 3. Glutathione peroxidase 1 (GPx1) is the most abundant version, found in the cytoplasm of nearly all mammalian tissues, whose preferred substrate is hydrogen peroxide. So far, eight different isoforms of glutathione peroxidase (GPx1-8) have been identified in humans.

Functions

Glutathione peroxidase belongs to the family of peroxidases. It protects the organism from oxidative damage. Its biochemical
<table>
<thead>
<tr>
<th>Isozyme</th>
<th>Gene</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione peroxidase 1</td>
<td>GPX1</td>
<td>Humans</td>
<td>Detoxification of H₂O₂</td>
</tr>
<tr>
<td>Glutathione peroxidase 2</td>
<td>GPX2</td>
<td>Humans¹</td>
<td>Detoxification of H₂O₂</td>
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<tr>
<td>Glutathione peroxidase 3</td>
<td>GPX3</td>
<td>Humans²</td>
<td>Detoxification of H₂O₂</td>
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<td>Glutathione peroxidase 4</td>
<td>GPX4</td>
<td>Mammals</td>
<td>Protects cells from lipid peroxidation</td>
</tr>
<tr>
<td>Glutathione peroxidase 5</td>
<td>GPX5</td>
<td>Mammals³</td>
<td>Protects spermatozoa</td>
</tr>
<tr>
<td>Glutathione peroxidase 6</td>
<td>GPX6</td>
<td>Humans⁴</td>
<td>Detoxification of H₂O₂</td>
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<tr>
<td>Glutathione peroxidase 7</td>
<td>GPX7</td>
<td>Humans</td>
<td>Alleviates oxidative stress</td>
</tr>
<tr>
<td>Glutathione peroxidase 8</td>
<td>GPX8</td>
<td>Putative</td>
<td>Detoxification of H₂O₂</td>
</tr>
</tbody>
</table>

¹ Gastrointestinal, ² Plasma, ³ Epididymis, ⁴ Olfactory

Table 3. Details of isoenzymes of glutathione peroxidase (GPxs).

functions are (i) to reduce free hydrogen peroxide to water and (ii) to reduce lipid hydroperoxides to their corresponding alcohols.

Structure

Glutathione peroxidase is a tetramer and has four identical polypeptide chains. Each unit has 178 amino acid residues in the form of two parallel and two anti-parallel pleated β-sheets surrounded by four α-helices.

Mechanism

Glutathione peroxidase is a remarkable enzyme in that it contains selenium atom in the form of selenocysteine very much required for its activity.

Catalytic reduction of hydrogen peroxide by glutathione peroxidase (GPx) is shown in Figure 8.

Glutathione peroxidase is a remarkable enzyme in that it contains selenium atom in the form of selenocysteine very much required for its activity. The active site contains the selenium analogue of
cysteine, in which Se has replaced sulphur. The selenolate (E-Se-)
form of this residue reduces the peroxide substrate to alcohol
and is, in turn, oxidized to selenic acid (E-Se-OH). Glutathione
now comes into action by forming a selenosulfide adduct (E-Se-
SG). The second glutathione then generates the active form of
the enzyme by attacking the selenosulphide to from oxidized gluta-
thione (GS-SG).

Generally, the activity of metalloenzymes [eg., catalase (Fe), per-
oxidase (Fe), superoxide dismutase (Cu)] depends on the pres-
ence of metal ions. It is puzzling to note that this particular en-
zeyme relies on a non-metal, i.e., selenium for its activity. The
mechanism starts with the oxidation of the selenol of a seleno-
cysteine residue by H₂O₂. The reactions are summarized below.

\[
R\text{SeH} + H_2O_2 \rightarrow R\text{SeOH} + H_2O \quad (4)
\]

\[
R\text{SeOH} + \text{GSH} \rightarrow \text{GS-} \text{SeR} + H_2O \quad (5)
\]

\[
\text{GS-} \text{SeR} + \text{GSH} \rightarrow \text{GS-SG} + \text{RSeH} \quad (6)
\]

The modulation of cellular reactive oxygen by metallo-enzymes

**Figure 8.** Reduction of hydrogen peroxide by glutathione peroxidase (GPx).
Figure 9. Modulation of cellular reactive oxygen species by metalloenzymes.

is shown in Figure 9.

In the terminal step, glutathione reductase reduces the oxidized glutathione to complete the cycle.

\[ \text{GS-SG + NADPH + H}^+ \rightarrow \text{2GSH + NADP}^+ \] (7)

Deficiency of glutathione peroxidase causes vitiligo disease, type 2 diabetes, and sclerosis.

Summary and Conclusions

Structure, function, and mechanism of action of metal-free enzymatic antioxidants, viz. thioredoxins, peroxiredoxins, glutathione S-transferases, and glutathione peroxidases are focused in this article. The unique features of these metal-free enzymes are highlighted. The chemistry of metalloenzymatic antioxidants, viz. superoxide dismutase (SOD), catalase (CAT), and peroxidases was delineated in the first part of the article. In this second article, the biochemistry of metal-free antioxidant enzymes, viz. thioredoxin, peroxiredoxin, glutathione S-transferases, and glutathione peroxidase is focused. It is of interest to compare metalloenzymatic antioxidants with metal-free enzymatic antioxidants. Metal-free antioxidant enzymes occur in several different isoforms and are more widely distributed than metalloenzymatic antiox-
idants. The antioxidant activity of metalloenzymes depends on metal ions as they serve as electron donors/acceptors and structural regulators. In metal-free antioxidant enzymes, sulfhydryl (–SH) groups of the proteins act as the electron donors or acceptors active sites.

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Suggested Reading