Chapter 1

Antioxidant Enzymes and Human Health

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1. Introduction

During normal metabolic functions, highly reactive compounds called free radicals are generated in the body; however, they may also be introduced from the environment. These molecules are inherently unstable as they possess lone pair of electrons and hence become highly reactive. They react with cellular molecules such as proteins, lipids and carbohydrates, and denature them. As a result of this, vital cellular structures and functions are lost and ultimately resulting in various pathological conditions.

Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals. For the past decade, countless studies have been devoted to the beneficial effects of antioxidant enzymes. It has been found that a substantial link exists between free radicals and more than sixty different health conditions, including the aging process, cancer, diabetes, Alzheimer's disease, strokes, heart attacks and atherosclerosis. By reducing exposure to free radicals and increasing the intake of antioxidant enzyme rich foods or antioxidant enzyme supplements, our body's potential to reducing the risk of free radical related health problems is made more palpable [1]. Antioxidant enzymes are, therefore, absolutely critical for maintaining optimal cellular and systemic health and well being. This chapter reviews the pathophysiological role of some of the important enzymes involved in free radical scavenging with their clinical applications.

2. Free radicals and their scavengers

Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized,

another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated, thousands of free radical reactions can occur within seconds of the initial reaction.

The ability of the cell to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called free radical or reactive oxygen species (ROS). About 5% or more of the inhaled O_2 is converted to ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals by univalent reduction of O_2.[2] Thus cells under aerobic condition are always threatened with the insult of ROS, which however are efficiently taken care of by the highly powerful antioxidant systems of the cell without any untoward effect. This antioxidant system includes, antioxidant enzymes (e.g., SOD, GPx and reductase, CAT, etc.), nutrient-derived antioxidants (e.g., ascorbic acid, tocopherols and tocotrienols, carotenoids, glutathione and lipoic acid), metal binding proteins (e.g., ferritin, lactoferrin, albumin, and ceruloplasmin) and numerous other antioxidant phytonutrients present in a wide variety of plant foods. Whenever the balance between ROS production and antioxidant defence is lost, 'oxidative stress' results which through a series of events deregulates the cellular functions leading to various pathological conditions.[3,4]

3. Reactive Oxygen Species

Reactive oxygen species (ROS) is a term that encompasses all highly reactive, oxygen containing molecules, including free radicals. Types of ROS include the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage. ROS are generated by a number of pathways. Most of the oxidants produced by cells occur as:

- A consequence of normal aerobic metabolism: approximately 90% of the oxygen utilized by the cell is consumed by the mitochondrial electron transport system.
- Oxidative burst from phagocytes (white blood cells) as part of the mechanism by which bacteria and viruses are killed, and by which foreign proteins (antigens) are denatured.
- Xenobiotic metabolism, i.e., detoxification of toxic substances.

Consequently, things like vigorous exercise, which accelerates cellular metabolism; chronic inflammation, infections, and other illnesses; exposure to allergens and the presence of "leaky gut" syndrome; and exposure to drugs or toxins such as cigarette smoke, pollution, pesticides, and insecticides may all contribute to an increase in the body's oxidant load.

3.1. Consequences of generation of ROS

Although O_2 can behave like a radical (a diradical) owing to presence of two unpaired electrons of parallel spin, it does not exhibit extreme reactivity due to quantum mechanical

restrictions. Its electronic structure result in formation of water by reduction with four electrons, i.e.:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

In the sequential univalent process by which O_2 undergoes reduction, several reactive intermediates are formed, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the extremely reactive hydroxy radical (°OH): collectively termed as the reactive oxygen species, the process can be represented as:

$$O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} {}^\circ OH \xrightarrow{e^-} H_2O$$

For the production of O_2^-, normally the tendency of univalent reduction of O_2 in respiring cells is restricted by cytochrome oxidase of the mitochondrial electron transport chain, which reduces O_2 by four electrons to H_2O without releasing either O_2^- or H_2O_2. However, O_2^- is invariably produced in respiring cells. This is due to the probable leak of single electron at the specific site of the mitochondrial electron transport chain, resulting in the appropriate single electron reduction of oxygen to O_2^-. When the electron transport chain is highly reduced, and the respiratory rate is dependent on ADP availability; leakage of electrons at the ubisemiquinone and ubiquinone sites increases so as to result in production of O_2^- and H_2O_2.

For the production of H_2O_2, peroxisomal oxidases and flavoprotein, as well as D-amino acid oxidase, L-hydroxy acid oxidase, and fatty acyl oxidase participate. Cytochrome P-450, P-450 reductase and cytochrome b-5 reductase in the endoplasmic reticulum under certain conditions generate O_2^-, and H_2O_2. During their catalytic cycles, likewise, the catalytic cycle of xanthine oxidase has emerged as important source of O_2^- and H_2O_2 in a number of different tissue injuries.

Finally, for the production of °OH, except during abnormal exposure to ionization radiation, generation of °OH in vivo requires the presence of trace amount of H_2O_2 and Fe^{2+} salt forms °OH, as given following Fenton reaction: [2]

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{2+} + {}^\circ OH + OH^-$$

Reactive oxygen species can attack vital cell components like polyunsaturated fatty acids, proteins, and nucleic acids. To a lesser extent, carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein synthesis, DNA damage; ultimately resulting in cell death (fig.01).[2]

Damage to cells caused by free radicals is believed to play a central role in various human disorders like rheumatoid arthritis, hemorrhagic shock, cardiovascular disease, cystic fibrosis, metabolic disorders, neurodegenerative disease, gastrointestinal ulcerogenesis, and AIDS. Some specific examples of ROS mediated disease are Alzheimer's disease, Parkinson's disease, oxidative modification of low-density lipoprotein in atherosclerosis, cancer, Down's syndrome, and ischemic reperfusion injury in different tissues including

heart, brain, kidney, liver, and gastrointestinal tract. Among these, role of ROS in atherosclerosis and ischemic injury in heart and brain studied extensively. [2,3]

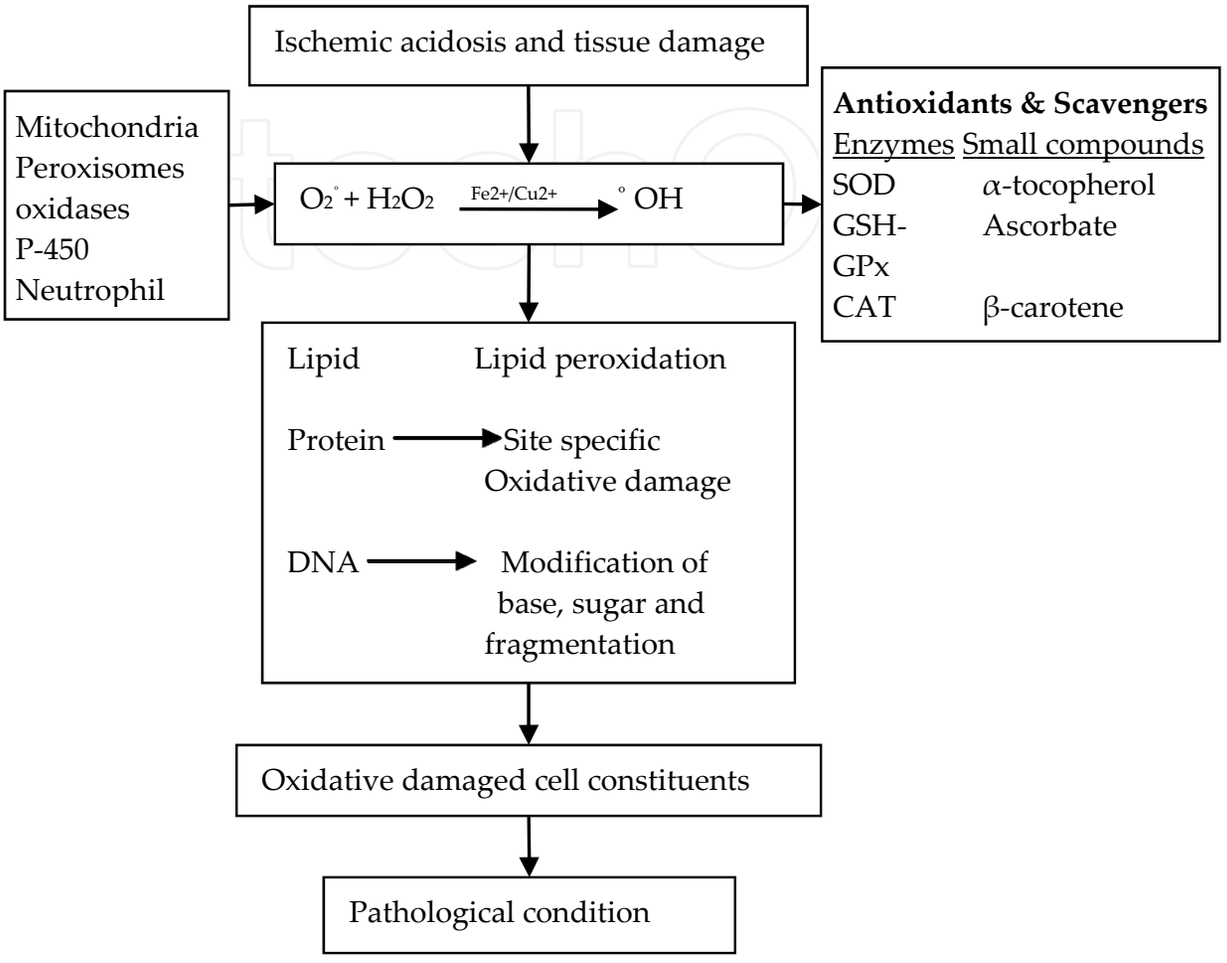

Figure 1. An overall picture of the metabolism of ROS and the mechanism of oxidative tissue damage leading to pathological conditions

4. Antioxidant protection system

To protect the cells and organ systems of the body against reactive oxygen species (ROS), humans have evolved a highly sophisticated and complex antioxidant protection system. It involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals (Table 1)[5] These components include:

a. Endogenous Antioxidants
   - Bilirubin
   - Thiols, e.g., glutathione, lipoic acid, N-acetyl cysteine
   - NADPH and NADH
   - Ubiquinone (coenzyme Q10)
   - Uric acid

- Enzymes:
  - copper/zinc and manganese-dependent superoxide dismutase
  - iron-dependent catalase
  - selenium-dependent glutathione peroxidase

b. Dietary Antioxidants
- Vitamin C
- Vitamin E
- Beta carotene and other carotenoids and oxycarotenoids, e.g., lycopene and lutein
- Polyphenols, e.g., flavonoids, flavones, flavonol's, and Proanthocyanidins

c. Metal Binding Proteins
- Albumin (copper)
- Ceruloplasmin (copper)
- Metallothionein (copper)
- Ferritin (iron)
- Myoglobin (iron)
- Transferrin (iron)

ROS	NEUTRALIZING ANTIOXIDANTS
Hydroxyl radical	Vitamin C, Glutathione Flavonoids, Lipoic acid
Superoxide radical	Vitamin C, Glutathione, Flavonoids, SOD
Hydrogen peroxide	Vitamin C, Glutathione, beta carotene, Vitamin-E, flavonoids, lipoic acid
Lipid peroxides	Beta-carotene, Vitamin-E, Ubiquinone, flavonoids, Glutathione peroxidase

Table 1. Various ROS and corresponding neutralizing antioxidants

Defence mechanisms against free radical-induced oxidative damage include the following:

i. catalytic removal of free radicals and reactive species by factors such as CAT, SOD, GPx and thiol-specific antioxidants;
ii. binding of proteins (e.g., transferrin, metallothionein, haptoglobins, caeroplasmin) to pro-oxidant metal ions, such as iron and copper;
iii. protection against macromolecular damage by proteins such as stress or heat shock proteins; and
iv. reduction of free radicals by electron donors, such as GSH, vitamin E (α- tocopherol), vitamin C (ascorbic acid), bilirubin, and uric acid [6]

Animal CAT are heme-containing enzymes that convert hydrogen peroxide (H_2O_2) to water and O_2, and they are largely localized in subcellular organelles such as peroxisomes. Mitochondria and the endoplasmic reticulum contain little CAT. Thus, intracellular H_2O_2 cannot be eliminated unless it diffuses to the peroxisomes [6]. GSH-Px removes H_2O_2 by coupling its reduction with the oxidation of GSH. GSH-Px can also reduce other peroxides, such as fatty acid hydro peroxides. These enzymes are present in the cytoplasm at

millimolar concentrations and also present in the mitochondrial matrix. Most animal tissues contain both CAT and GSH-Px activity.

SODs are metal-containing proteins that catalyze the removal of superoxide, generating water peroxide as a final product of the dismutation. Three isoforms have been identified, and they all are present in all eukaryotic cells. The copper-zinc SOD isoform is present in the cytoplasm, nucleus, and plasma. On the other hand, the manganese SOD isoform is primarily located in mitochondria.

Dietary micronutrients also contribute to the antioxidant defence system. These include β -carotene, vitamin C, and vitamin E (the vitamin E family comprises both tocopherols and tocotrienols, with α- tocopherol being the predominant and most active form). Water-soluble molecules, such as vitamin C, are potent radical scavenging agents in the aqueous phase of the cytoplasm, whereas lipid soluble forms, such as vitamin E and β- carotene, act as antioxidants within lipid environments. Selenium, copper, zinc, and manganese are also important elements, since they act as cofactors for antioxidant enzymes. Selenium is considered particularly important in protecting the lipid environment against oxidative injury, as it serves as a cofactor for GSH-Px [6–8].

The most abundant cellular antioxidant is the tripeptide, GSH(l-L-γ-glutamyl-l-cysteinyl glycine). GSH is synthesized in two steps. First, γ-glutamyl cysteine synthetase (γ-GCS) forms a γ-peptide bond between glutamic acid and cysteine, and then GSH synthetase adds glycine. GSH prevents the oxidation of protein thiol groups, either directly by reacting with reactive species or indirectly through glutathione transferases [6-8].

5. Antioxidant enzymes in health

Antioxidants are of different types so that they might be available for action when and where they are needed. They are natural (enzymes antioxidants and metal carrier proteins in the body), scavenging or chain breaking (like vitamin A, C, beta-carotene, etc.), pharmacologic antioxidants and others. Antioxidant compounds must be up'' (converted) in the process of neutralizing free radicals. Therefore, one must continually produce more of the antioxidants in the body or ingest them either in diet or by supply mentation. The repair enzymes that can regrate some antioxidants are SOD, GPx, glutathione reductase (GR), CAT and the other metalloenzymes.

SOD, CAT, and GPx constitute a mutually supportive team of defence against ROS. While SOD lowers the steady-state level of O^{2-}, catalase and peroxidases do the same for H_2O_2.

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

$$ROOH / H_2O_2 \xrightarrow{\text{GSH-Peroxidase}} ROH / H_2O + GSSG + GSH$$

$$H_2O_2 + AH_2 \xrightarrow{\text{Peroxidase}} 2H_2O + A$$

$$H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

Catalytic removal of ROS by antioxidant enzyme

Endogenous Antioxidants

In addition to dietary antioxidants, the body relies on several endogenous defence mechanisms to help protect against free radical-induced cell damage. The antioxidant enzymes – GPx, heme peroxidase, CAT, and SOD – metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity. Glutathione, an important water-soluble antioxidant, is synthesized from the amino acids glycine, glutamate, and cysteine. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism. Exposure of the liver to xenobiotic substances induces oxidative reactions through the up regulation of detoxification enzymes, i.e., cytochrome P-450 mixed-function oxidase. When an individual is exposed to high levels of xenobiotics, more glutathione is utilized for conjugation (a key step in the body's detoxification process) making it less available to serve as an antioxidant. Research suggests that glutathione and vitamin C work interactively to quench free radicals and that they have a sparing effect upon each other. Lipoic acid, yet another important endogenous antioxidant, categorized as a "thiol" or "biothiol," is a sulphur-containing molecule that is known for its involvement in the reaction that catalyzes the oxidative decarboxylation of alpha-keto acids, such as pyruvate and alphaketoglutarate, in the Krebs cycle. Lipoic acid and its reduced form, dihydrolipoic acid (DHLA), are capable of quenching free radicals in both lipid and aqueous domains and as such has been called a "universal antioxidant." Lipoic acid may also exert its antioxidant effect by cheating with pro-oxidant metals. Research further suggests that lipoic acid has a sparing effect on other antioxidants. Animal studies have demonstrated supplemental lipoic acid to protect against the symptoms of vitamin E or vitamin C deficiency.

Superoxide dismutase

In 1967 biochemist Irwin Fridovitch of Duke University and Joe McCord discovered the antioxidant enzyme SOD, which provides an important means of cellular defence against free radical damage. This breakthrough caused medical scientists to begin to look seriously at free radicals. In most cases the process is automatically controlled and the number of free radicals does not become dangerously high. Fortunately, the body has, throughout the course of millions of years of evaluation become accustomed to coping with free radicals and has evolved various schemes for doing this [3].

SOD (EC 1.15.1.1) is the antioxidant enzyme that catalysed the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. Peroxide can be destroyed by CAT or GPX reactions [9-11].

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

In humans, there are three forms of SOD: cytosolic Cu/Zn-SOD, mitochondrial Mn-SOD, and extracellular SOD (EC-SOD) [12,13]. SOD destroys O_2^- by successive oxidation and

reduction of the transition metal ion at the active site in a Ping Pong type mechanism with remarkably high reaction rates [14]. All types of SOD bind single charged anions such as azide and fluoride, but distinct differences have been noted in the susceptibilities of Fe-, Mn^- or Cu/Zn-SODs. Cu/Zn-SOD is competitively inhibited by N_3^- , CN^- [15], and by F^- [16].

Mn-SOD is a homotetramer (96 kDa) containing one manganese atom per subunit those cycles from Mn (III) to Mn (II) and back to Mn (III) during the two step dismutation of superoxide [17]. The respiratory chain in mitochondria is a major source of oxygen radicals. Mn-SOD has been shown to be greatly induced and depressed by cytokines, but is only moderately influenced by oxidants [17]. Inactivation of recombinant human mitochondrial Mn- SOD by peroxynitrite is caused by nitration of a specific tyrosine residue [18].

The biological importance of Mn-SOD is demonstrated among others by the following observations: (a) inactivation of Mn-SOD genes in *Escherichia coli* increases mutation frequency when grown under aerobic conditions [19]; (b) elimination of the gene in *Saccharomyces cerevisiae* increases its sensitivity to oxygen [20], (c) lack of expression in Mn-SOD knockout mice results in dilated cardiomyopathy and neonatal lethality [21]; (d) tumor necrosis factor (TNF) selectively induces Mn-SOD, but not Cu/Zn- SOD, CAT or GPX mRNA in various mouse tissues and cultured cells [22,23]; (e) transection of Mn- SOD cDNA into cultured cells rendered the cells resistant to parquet, TNF and Adriamycin-induced cytotoxicity, and radiation induced-neoplastic transformation [24]; f) expression of human Mn-SOD genes in transgenic mice protects against oxygen induced pulmonary injury and Adriamycin-induced cardiac toxicity [25].

Cu/Zn-SOD (SOD-1) is another type of enzymes that has been conserved throughout evolution. These enzymes have two identical subunits of about 32 kDa, although a monomeric structure can be found in a high protein concentration from *E. coli* [26]. Each subunit contains a metal cluster, the active site, constituted by a copper and a zinc atom bridged by a histamine residue [27,28,29].

Cu/Zn-SOD is believed to play a major role in the first line of antioxidant defence. Calves that were fed milk supplemented with 25 ppm Cu and 100 ppm Zn showed a stronger immune response and a higher SOD activity [30]. Other recent reports involving SOD knock-outs have revealed that Mn- SOD is essential for life whereas Cu/Zn-SOD is not. Cu/Zn-SOD knock-out mice appear normal and exhibit differences only after traumatic injury, whereas Mn-SOD knockouts do not survive past 3 weeks of age [31]. Among various human tissues Mn-SOD contents were roughly one-half as large as the Cu/Zn-SOD contents [31]. Extracellular superoxide dismutase (EC-SOD) is a secretory, tetrameric, copper and zinc containing glycoprotein; with a high affinity for certain glycosaminoglycans such as heparin and heparin sulphate. EC-SOD was found in the interstitial spaces of tissues and also in extracellular fluids, accounting for the majority of the SOD activity in plasma, lymph, and synovial fluid. EC-SOD is not induced by its substrate or by other oxidants and its regulation in mammalian tissues primarily occurs in a manner coordinated by cytokines, rather than as a response of individual cells to oxidants [32].

Application

This enzyme has been known to promote the rejuvenation and repair of cells, while reducing the damages caused by free radicals. SOD is found in our skin and it is essential in order for our body to generate adequate amounts of skin-building cells called fibroblasts. Among the common natural sources of SOD are cabbage, Brussels sprouts, wheat grass, barley grass and broccoli. SOD plays a significant role in preventing the development of the Lou Gehrig's disease, also known as Amyotrophic Lateral Sclerosis (ALS). This kind of illness can lead to death because it affects the nerve cells in the spinal cord and the brain. Apart from that, this enzyme is also used for treatment of inflammatory diseases, burn injuries, prostate problems, arthritis, corneal ulcer, and reversing the long term effects of radiation and smoke exposure. Additionally, if superoxide dismutase is made into a lotion and applied to the skin, it will prevent the formation of wrinkles. It will also heal wounds, reduce the appearance of scars, and lighten skin pigmentation that has been caused by UV rays.

SOD is also known to help carry nitric oxide into our hair follicles. This is beneficial for people who are experiencing premature hair loss due to a genetic predisposition or free radicals. Because this enzyme is a very potent antioxidant, SOD combats the effects of free radicals that are causing hair follicles to die. Since nitric oxide relaxes the blood vessels and allows more blood to circulate to the hair follicles and SOD helps to remove the free radicals, hair loss can be prevented and even reversed. Taking dietary supplements that provide an adequate supply of Superoxide dismutase will be helpful in maintaining overall well being and health because it protects our entire body from the harmful effects of free radicals.

Catalase

Catalase (CAT) is an enzyme responsible for the degradation of hydrogen peroxide. It is a protective enzyme present in nearly all animal cells.

Specificity

The reaction of CAT occurs in two steps. A molecule of hydrogen peroxide oxidizes the heme to an oxyferryl species. A porphyrin cation radical is generated when one oxidation equivalent is removed from iron and one from the porphyrin ring. A second hydrogen peroxide molecule acts as a reducing agent to regenerate the resting state enzyme, producing a molecule of oxygen and water.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

$$ROOH + AH_2 \longrightarrow H_2O + ROH + A$$

CAT (EC 1.11.1.6) is a tetrameric enzyme consisting of four identical tetrahedrally arranged subunits of 60 kDa that contains a single ferriprotoporphyrin group per subunit, and has a molecular mass of about 240 kDa [33]. CAT reacts very efficiently with H_2O_2 to form water and molecular oxygen; and with H donors (methanol, ethanol, formic acid, or phenols) with peroxidase activity.

In animals, hydrogen peroxide is detoxified by CAT and by GPX. CAT protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells. Survival of rats exposed to 100% oxygen was increased when liposome's containing SOD and CAT were injected intravenously before and during the exposure [34]. The increased sensitivity of transfected CAT-enriched cells to some drugs and oxidants is attributed to the property of CAT in cells to prevent the drug-induced consumption of O2 either for destroying H2O2 to oxygen or for direct interaction with the drug [35].

Application

CAT is used in the food industry for removing hydrogen peroxide from milk prior to cheese production. Another use is in food wrappers where it prevents food from oxidizing CAT is also used in the textile industry, removing hydrogen peroxide from fabrics to make sure the material is peroxide-free. A minor use is in contact lens hygiene - a few lens-cleaning products disinfect the lens using a hydrogen peroxide solution; a solution containing CAT is then used to decompose the hydrogen peroxide before the lens is used again. Recently, CAT has also begun to be used in the aesthetics industry. Several mask treatments combine the enzyme with hydrogen peroxide on the face with the intent of increasing cellular oxygenation in the upper layers of the epidermis.

Glutathione peroxidase

Glutathione peroxidase (GPx) is an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides.

The GPx (EC 1.11.1.19) contains a single selenocysteine selenocysteine (Sec) residue in each of the four identical subunits, which is essential for enzyme activity [36]. GPX (80 kDa) catalyses the reduction of hydro peroxides using GSH, thereby protecting mammalian cells against oxidative damage. In fact, glutathione metabolism is one of the most essential antioxidative defence mechanisms.

$$ROOH + 2GSH \longrightarrow ROH + GSSG + H_2O$$

There are five GPx isoenzymes found in mammals. Although their expression is ubiquitous, the levels of each isoform vary depending on the tissue type. Cytosolic and mitochondrial glutathione peroxidase (cGPX or GPX1) reduces fatty acid hydroperoxides and H_20_2 at the expense of glutathione. GPX1 and the phospholipid hydroperoxide glutathione peroxidase (PHGPX or GPX4) are found in most tissues. GPX4 is located in both the cytosol and the membrane fraction. PHGPX can directly reduce the phospholipid hydroperoxides, fatty acid hydroperoxides, and cholesterol hydroperoxides that are produced in peroxidized membranes and oxidized lipoproteins [37]. GPX1 is predominantly present in erythrocytes, kidney, and liver, and GPX4 is highly expressed in renal epithelial cells and testes. Cytosolic GPX2 or GPX-G1, and extracellular GPX3 or GPX-P is poorly detected in most tissues except for the gastrointestinal tract and kidney, respectively. Recently, a new member, GPX5,

expressed specifically in mouse epididymis, is interestingly selenium-independent [38]. Although GPX shares the substrate, H_2O_2, with CAT, it alone can react effectively with lipid and other organic hydroperoxides, being the major source of protection against low levels of oxidant stress.

Application

This is one of the most important enzymes in the body with antioxidant properties. Levels of GPx in the body are closely linked with that of glutathione, the master antioxidant. Glutathione (GHS for short) is a tripeptide that not only protects the cells against ill effects of pollution; it is also acts as your body's immune system boosters. It is present in high concentrations in the cells and plays a pivotal role in maintaining them in reduced state lest they suffer damage by oxidation (from free radicals). The role as antioxidant is particularly important for brain as it is very sensitive to presence of free radicals. Combination of certain antioxidants like glutathione, vitamin C and E, selenium and glutathione peroxidase are very powerful in helping the body fight against the free radicals. GSH ensures that the red blood cells remain intact and protect the white blood cells (which are responsible for immunity). Glutathione is found in vegetables and fruit, but cooking will significantly reduce its potency. Taking it as a supplement is a good idea.

6. Clinical applications of antioxidant enzymes

1. **Chronic Inflammation:** Chronic inflammatory diseases such as rheumatoid arthritis are self-perpetuated by the free radicals released by neutrophils. Both corticosteroids and non-steroids anti inflammatory drugs interfere with formation of free radicals and interrupt the disease process.
2. **Acute Inflammation:** At the inflammatory site, activated macrophages produce free radicals. Respiratory burst and increased activity of NADPH oxidase are seen in macrophages and neutrophils.
3. **Respiratory Diseases:** Breathing of 100 % oxygen for more than 24 hr produces destruction of endothelium and lung edema. This is due to the release of free radicals by activated neutrophils [39].

   In premature newborn infants, prolonged exposure to high oxygen concentration is responsible for bronchopulmonary dysplasia. Adult respiratory distress syndrome (ARDS) is characterized by pulmonary edema. ARDS is produced when neutrophils are recruited to lungs which subsequently release free radicals.

   Cigarette smoking enhances the emphysema in alpha-1 protease inhibitor deficiency. Cigarette smoke contains free radicals. Soot attracts neutrophils to the site which releases more free radicals. Thus, there is more elastase and less protease inhibitor, leading to lung damage.

4. **Diseases of the Eye:** Retrolental fibroplasia or retinopathy of prematurity is a condition seen in premature infants treated with pure oxygen for a long time. It is caused by free

radicals, causing thromboxane release, sustained vascular contracture and cellular injury. Cataract formation is related with ageing process. Cataract is partly due to photochemical generation of free radicals. Tissues of the eye, including the lens, have high concentration of free radical scavenging enzymes.

5. **Shock Related Injury:** Release of free radicals from phagocytes damage membranes by lipid peroxidation. They release leucotrienes from platelets and proteases from macrophages. All these factors cause increased vascular permeability, resulting in tissue edema. Anti-oxidants have a protective effect.
6. **Arthrosclerosis and Myocardial Infraction:** Low density lipoproteins (LDL) promote atherosclerosis. They are deposited under the endothelial cells, which undergo oxidation by free radicals released from endothelial cells. This attracts macrophages. Macrophages are them converted into foam cells. This initiates the atherosclerotic plaque formation. Alpha tocopherol offers some protective effect.
7. **Peptic Ulcer:** Peptic ulcer is produced by erosion of gastric mucosa by hydrochloric acid. It is shown that superoxide anions are involved in the formation of ulcer. Helicobacter pylori infection perpetuates the disease. This infection potentiates the macrophage oxidative burst leading to tissue destruction.
8. **Skin Diseases:** due to inborn defects, porphyrins accumulate in the skin. Exposure of sunlight will lead to erythema and eruptions in the patients. Sunlight acting on porphyrins produces singlet oxygen, which trigger inflammatory reaction, leading to the above symptoms. Certain plant products, called psoralens are administered in the treatment of psoriasis and leukoderma. When the drugs is applied over the affected skin and then irradiated by UV light, singlet oxygen produced with clinical benefit.
9. **Cancer Treatment [39]:** Free radicals contribute to cancer development because of their mutagenic property. Free radicals produce DNA damage, and accumulated damages lead to somatic mutations and malignancy. Cancer is treated by radiotherapy. Irrational produces reactive oxygen species in the cells which trigger the cell death. To increase the therapeutic effect of radiation, radio-sensitisers are administered, which increase the production of ROS.

7. Other antioxidants

Dietary Antioxidants

Vitamin C, vitamin E, and beta-carotene are among the most widely studied dietary antioxidants. Vitamin C is considered the most important water-soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is initiated. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation. Vitamin C has been cited as being capable of regenerating vitamin E.

Beta-carotene and other carotenoids are also believed to provide antioxidant protection to lipid-rich tissues. Research suggests beta-carotene may work synergistically with vitamin E.

A diet that is excessively low in fat may negatively affect beta carotene and vitamin E absorption, as well as other fat-soluble nutrients. Fruits and vegetables are major sources of vitamin C and carotenoids, while whole grains and high quality, properly extracted and protected vegetable oils are major sources of vitamin E. [5]

Phytonutrients

A number of other dietary antioxidant substances exist beyond the traditional vitamins discussed above. Many plant-derived substances, collectively termed "phytonutrients," or "phytochemicals," are becoming increasingly known for their antioxidant activity. Phenolic compounds such as flavonoids are ubiquitous within the plant kingdom: approximately 3,000 flavonoid substances have been described. In plants, flavonoids serve as protectors against a wide variety of environmental stresses while, in humans, flavonoids appear to function as "biological response modifiers." Flavonoids have been demonstrated to have anti-inflammatory, antiallergenic, anti-viral, anti-aging, and anti-carcinogenic activity. The broad therapeutic effects of flavonoids can be largely attributed to their antioxidant properties. In addition to an antioxidant effect, flavonoid compounds may exert protection against heart disease through the inhibition of cyclooxygenase and lipoxygenase activities in platelets and macrophages. [5]

8. Conclusion

Oxidative stress plays a major role in the pathogenic of many disorders including aging, cancer, diabetes, alzheimer's, strokes, viral infections (that cause airway epithelial inflammation), neurodegenerative processes (including cell death, motor neuron diseases and axonal injury) and infraction, and brain edema. Antioxidant enzyme plays an important role in protecting oxidative injury to the body. One of the therapeutic approach by which these disorders can be prevented is to increase the levels of these enzymes (SOD, CAT, GPx etc.) in the body by interventions which may include increases intake of dietary supplements rich in antioxidants/antioxidant enzymes and regular exercise.

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