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Superoxide Dismutase, Structure Function and Mechanism

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Abstract

Living organisms have evolved protecting systems to protect themselves against oxidative stress and to prevent damage from these toxic agents. They have developed several enzymatic and non-enzymatic mechanisms to detoxify these very active compounds. Enzymatically, oxygen radicals are removed mainly by the action of four enzymes: superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase. Superoxide dismutases (SODs) are metalloenzymes that catalyze the conversion of superoxide molecules to hydrogen peroxide and molecular oxygen and therefore form one of the cell's major defense mechanisms against oxidative stress. This review will discuss superoxide dismutase, structure function and mechanism

Keywords: Superoxide dismutase; Free radicals; Oxidative stress; Enzymes; Metalloenzymes.

Introduction

Molecular oxygen (O_2) is essential for the aerobic life on Earth. All aerobic organisms undergo complete reduction of molecular oxygen (O_2) and generate energy in the form of ATP which is used to carry out biological functions. Molecular oxygen (O_2), while crucial for the life of aerobes, is potentially toxic as when it is incompletely reduced, it produces some reactive intermediates such as hydrogen peroxide (H_2O_2), superoxide anion radical (O_2^-), and the highly reactive hydroxyl radicals ($^{\bullet}OH$) and these oxy-radicals are referred to as reactive oxygen species (Fridovich, 2004). Superoxide radicals and other oxy-radical intermediates are easily formed e.g. by autooxidation and this is an unavoidable event in aerobic respiration. These reactive oxygen species (ROS) are essential for various functions such as homeostasis and cell signaling but an imbalance in favour of reactive oxygen species results in oxidative stress (OS) (Kashmiri *et al.*, 2014). Oxidative stress (OS) result in interference in the functioning of biological systems that maintain levels of environmentally produced reactive oxygen species (ROS), by readily detecting and detoxifying them (Lucana *et al.*, 2012). Reactive oxygen species rapidly react with various molecules and interfere with cellular functions and causes oxygen toxicity. This toxicity is due to the tendency of O_2 for reduction by a univalent pathway. This simplistic univalent pathway of

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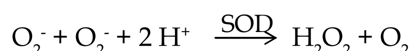
O_2 reduction generates intermediates that lie between one O_2 and its four electron reduction products – two molecules of water – and it is the reactivity of these intermediates that is responsible for the toxicity of O_2 (Fridovich, 2004). Therefore, protection of tissues from oxygen toxicity is one of the major requisites for aerobic life. Hence, to abate oxygen toxicity, the reactive species should be scavenged effectively at the site of generation.

Living organisms have evolved protecting systems to protect themselves against oxidative stress and to prevent damage from these toxic agents. They have developed several enzymatic and non-enzymatic mechanisms to detoxify these very active compounds. Enzymatically, oxygen radicals are removed mainly by the action of four enzymes: superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase (Yesilkaya *et al.*, 2000). Superoxide dismutases (SODs) are metalloenzymes that catalyze the conversion of superoxide molecules to hydrogen peroxide and molecular oxygen and therefore form one of the cell's major defense mechanisms against oxidative stress.

These antioxidant enzymes are very important and are widely distributed in prokaryotic and eukaryotic cells. They catalyze the reduction of the superoxide radical to hydrogen peroxide and dioxygen in a critical reaction i.e.,



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

That protects aerobic organisms against oxidative damage. Superoxide dismutases, as the name suggests dismutes superoxide. Dismutation reaction is that in which two equal but opposite reactions occur on two separate molecules. SOD takes two molecules of superoxide, removes the extra electron from one and add it to the other. At the end hydrogen peroxide (less damaging) and oxygen are formed (Fridovich, 1989). Hydrogen peroxide can then subsequently be reduced to water, or to water and molecular oxygen, by the action of other enzyme systems (Stromqvist, 1993).

Superoxide dismutases (SODs) have been classified into four families based on their different types of metal centers: copper/zinc, nickel, manganese and iron (Beyer *et al*., 1991). In prokaryotes, on the basis of metal cofactor, three types of SODs have been defined: Cu-Zn- (SodC), Fe- (SodB), or Mn-type (SodA) SODs. FeSOD and MnSOD are characteristically prokaryote enzymes, but MnSOD is also present in mitochondria of eukaryotes. Cu-ZnSOD, on the other hand, is mainly found in the cytosol of many eukaryotic organisms. However, several prokaryotes containing Cu-ZnSOD and Ni-SOD (Hammouda *et al*.,1999) have been reported.

There are many structural and chemical differences between bacterial and human SODs. Bacterial SODs do show novelties not found in eukaryotic dismutases. These novelties may suggest ways to engineer human dismutases or new ways to formulate on-going questions of clinical importance. MnSOD is present in both eukaryotic and prokaryotic cells and study of bacterial MnSOD can lead to the development of various therapies involving human MnSOD.

Oxidative stress

Aerobic organisms need molecular oxygen (O_2) for respiration process or nutrient oxidation to obtain energy but it is also a potentially very toxic agent because of its capability to form oxy radicals. Despite that it provides vast advantages, it also contains a universal toxicity (Fridovich, 1983). Numerous researches have reported oxygen toxicity in various species (Gottlieb, 1971; Wolfe and De Vries, 1975). It has been revealed to be mediated by products generated from the univalent reduction of molecular dioxygen, including the superoxide radical ($O_2^{\bullet}$), the hydroxyl radical ($OH^{\bullet}$) and the hydrogen peroxide (H_2O_2) (Fridovich, 1983; Halliwell and Gutteridge, 1984; Carlioz and Touati, 1986). Derived from molecular oxygen, these intermediates are by-products of cellular respiration that are produced continuously in cells growing aerobically, and are called reactive oxygen species (ROS). There are abundant sources of reactive oxygen intermediates which include partial reduction of oxygen during respiration, radiation exposure or exposure to redox-active compounds, and the burst of phagocytes during respiration (Yesilkaya *et al*., 2000). These reactive oxygen species (ROS) induces the oxidative stress (OS) (Kashmiri *et al.,* 2014). Normally ATP is generated from glucose and O_2 by cytochrome oxidase and in this process of generating ATP, O_2 is usually reduced to H_2O by four electrons. Infrequently (approximately 5% of the time), O_2 is reduced by single electron, yielding superoxide ($O_2^{\bullet}$). Further reduction of O_2 occurs by one or two additional electrons yielding hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), respectively. However, there are additional sources of these oxy-radicals such as the interaction of ionizing radiation with biological tissues and other metabolic processes (James, 1994).

Molecular oxygen (dioxygen) is comparatively unreactive in its ground state, yet it has the capability to give rise to fatal reactive excited states such as free radicals and their derivatives. A complete stepwise reduction process utilizes O_2; where four electrons reduce to water and during this incompletely reduced reactive intermediates are generated (Fig. 1).

Partially reduced reactive species comprises the superoxide radical ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\bullet}OH$). Thus, molecular oxygen, although crucial for the survival and existence of aerobic life, boons living organisms with a variety of biological challenges collectively called "oxidative stress." These reactive oxygen species leads to damage of proteins, nucleic acids and cell membranes as shown in Fig. 2. Increasing evidence suggests that the collective damage caused by these reactive oxygen species leads to numerous diseases.

Several enzymes are expressed constitutively by cells to suppress oxidative stress. The reactive oxygen species are detoxified by these enzymes and thus helping in the repair of damage produced by them. In addition, the different cells of bacteria, yeast and mammalian, all have adaptive responses to elevated levels of oxidative stress, showing that these cells detect the increased amount of reactive oxygen species and this signal is transduced into an enhanced expression of defensive activities (Storz and Imlay, 1999).

Superoxide dismutases

During 1970s, rapid growth and development was observed in the field of biology of free radicals and medicine. Many people considered the event of discovery of superoxide dismutase (SOD) to be responsible for this growth and development. The incident of discovery of SOD has been as often by chance and was made not by design (McCord and Fridovich, 1988). SOD (EC 1.15.1.1) was first extracted from bovine blood and was known to be a green copper protein (Mann and Keilin, 1938) and its function was thought to be of copper storage.

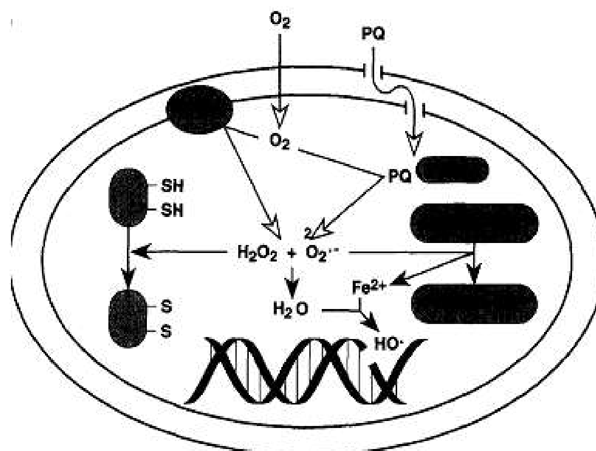


Fig. 2: Mechanism of oxidative cell damage by endogenous oxidants. Molecular oxygen passively diffuses into cells and is converted to •O2- and H2O2 by the direct oxidation of flavoproteins, including NADH dehydrogenase II (Ndh II). Redox-cycling drugs, including paraquat (PQ), accelerate the formation of these oxygen species by catalysing the transfer of electrons from redox enzymes such as sulphite reductase (SiRase) to oxygen. •O2- oxidatively destroys iron-sulfur clusters (here, from aconitase, Acn). The released iron can react with H2O2 to form hydroxyl radical •OH, which directly damages DNA (Storz and Imlay, 1999).

During several years after the discovery, the enzyme was variably known as erythrocuprein, indophenol oxidase, and tetrazolium oxidase (Scandalios, 1993). In 1968, at Duke University, Irwin Fridovich along with Joe McCord were the first to discover the enzymatic action of superoxide dismutase. Until this discovery, the actual function of SODs was not known and they were considered to be a group of proteins that have metal ions at

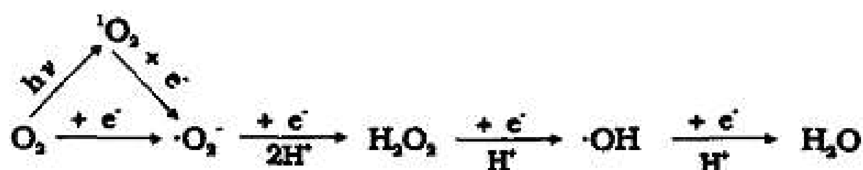
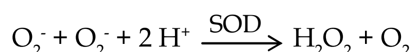


Fig. 1: Pathways showing the reduction of O_2 to water resulting in the formation of various intermediate reactive O_2 species (Scandalios, 1993).

their active centres i.e. metalloproteins. Later on, the manganese-containing (Mn)(Fridovich, 1970) and the iron-containing(Fe) (Fridovich, 1973) SODs from *E. coli* bacteria and the MnSOD (SOD_2) from mitochondria were also discovered by Fridovich and his research group. Mitochondrial MnSOD is now considered as a vital mammalian protein (Fridovich, 1988). Brewer in 1967 analyzed the proteins of starch gels using tphenazine-tetrazolium technique and identified a protein, indophenol oxidase, that later known as superoxide dismutase.

Superoxide dismutases (SODs) catalyze the dismutation reaction where conversion of O_2 into H_2O_2 and O_2 occurs, i.e.,



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

and by doing so, they provide a significant defense. The product of dismutation reaction, i.e. H_2O_2, is further eliminated by other defensive enzymes that include the catalases and peroxidases. In this way, the rigorous action of the SODs together with the catalases and peroxidases inhibits the formation of the very reactive hydroxyl radical (HO**)** (Fridovich, 2004).

Cells which respire and which can thus produce O_2^- must have evolved appropriate defenses against this radical. The underpinning of such defenses is provided by enzymes, which scavenge O_2^-, by catalyzing the above reaction. These enzymes, which have been termed as superoxide dismutases, are vital for the survival of respiring organisms. This enzyme has been identified in a widespread range of living things and has been considered as an important defense against the universal potential toxicity of oxygen. This statement applies to bacteria, algae, protozoa, fungi, plants, insects, birds and mammals (Fridovich, 1975).

Virtually, there are two main types of SOD present in different organisms that catalyze the dismutation of O_2^- and this occurs within different cell organelles and other cellular compartments. The SODs can be characterized on the basis of metal ions (Mn/Fe, Cu, and Zn) present at the active site of the enzyme. The presence of the superoxide dismutase enzyme also depends on the type of cell. Prokaryotes especially bacteria normally contain one type of SOD either Mn/Fe or Cu-Zn, whereas almost all eukaryotes contain both types. Superoxide dismutase enzyme is ubiquitous in nature. Though it is widely dispersed among oxygen consuming organisms, it is also found in aerotolerant anaerobes, and some obligate anaerobes (Fridovich, 1986).

Biological function of superoxide dismutases

The biological function and importance of SODs as defensive enzymes against O_2 toxicity have been exhibited in several studies with prokaryotes, eukaryotes both lower and higher, including higher plants (Fridovich, 1986; Hassan and Scandalios, 1990; Scandalios, 1990, 1992; Bowler et al., 1992; Gralla and Kosman, 1992). When the amount of SOD enzyme in cells decreases, it results in the increased generation of oxy-radicals. This imbalanced condition has been associated with wide-ranging pathological conditions such as inflammatory tissue necrosis, formation of cataract and aging, tumor development, asthma, drug-induced liver necrosis (comprising acetaminophen damage), and many neurodegenerative disorders (James, 1994).

The cumulative action of superoxide dismutase (SOD) and catalase (CAT) reduces the production of the most lethal and highly reactive oxidant that is the hydroxyl radical ($OH^\bullet$) (Scandalios, 1993). SOD and other related antioxidants are localized withinand around cells of damaged tissues in a way to prevent oxidative stress produced by superoxide andits metabolites (Inoue 1994).

Classification of superoxide dismutases

There are SODs that are classified on the basis of metal ion present at the active site, i.e. Cu (II) plus Zn (II), Mn (III), Fe (III), and Ni (II). They all catalyze the dismutation of O_2 into H_2O_2 and O_2. All SODs function in a similar manner where the metal at the active site is reduced by one O_2 molecule and then reoxidized by the next O_2 molecule. Thus the active metal centre acts like a mediator that passes an electron from one molecule of oxygen to another (Fridovich, 2004). As mentioned above, based on the metal centre there are four major families of SODs as follows:

1. *MN SODs* - This type of SOD enzyme is contained in both prokaryotes and eukaryotes. This enzyme, whether from bacteria or from the mitochondrial matrix, showed discernible sequence similarity, revealing a close evolutionary history and showing an endosymbiotic origin for mitochondria. There is some structural difference between bacterial and mitochondrial Mn SOD. The bacterial SOD is generally a homodimer, and the corresponding enzyme from mitochondria is a homotetramer. The weight of subunit of this enzyme is 23 kDa. Some bacterial enzymes are also

tetrameric as is the case of *Cryptococcus neoformans*.

2. CU, ZN SODs – This type of SOD is usually found in chloroplasts, the periplasm of gram-negative bacteria, cytosols of eukaryotic cells and in the intermembrane space of mitochondria. The bacterial enzyme found in eukaryotic cytosols is usually a homodimeric protein with subunit weight of ~16 kDa, whereas the enzyme from periplasm of *E. Coli* is monomeric protein. In higher animals, the extracellular Cu, Zn SOD is present. The extracellular Cu, Zn SOD is usually homotetrameric in structure and is glycosylated with subunit weighing ~ 23 kDa.

3. FE SODs – Fe SODs are highly homologous to the Mn SODs and are present in bacteria and in plants. Although Fe SODs are usually homodimeric proteins, homotetrameric Fe SOD has been detected in *Mycobacterum tuberculosis* and *Rhodococcus bronchialis*. The Fe SOD enzyme of *Escherichia coli* is constitutive so it is found even in cells grown anaerobically. It can thus be viewed as a stand by defense against O_2, which is always maintained to protect in the event of a sudden exposure to O_2 (Fridovich, 2004).

4. NI SODs – These SODs are found in prokaryotic organisms. The Ni SOD enzyme is hexameric protein and comprises of right-handed 4-helix bundles. Each right-handed helix bundle comprises of N-terminal hooks and these assist in chelating a Ni ion. The Ni-hook comprises of motif His-Cys-X-X-Pro-Cys-Gly-X-Tyr. This motif is accountable for most of the interactions that are critical for binding with metal and catalysis (Barondeau *et al*., 2004).

Bacterial superoxide dismutases

Superoxide dismutase enzyme is found in a variety of bacterial sources such as *Escherichia coli* and mammalian sources and it has been confirmed that it catalyzes the dismutation reaction where univalently reduced oxygen gets disproportionated (Keele *et al*., 1970). The mammalian enzyme contains copper and zinc and due to this it imparts blue colour, whereas the corresponding enzyme in bacteria was found to contain manganese and imparting red-purple colour. This enzyme when isolated from *E. coli* bacteria were found to have a molecular weight of 39,500 daltonand this was determined by sedimentation equilibrium technique. The sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of the protein showed that this enzyme comprises of two subunits having equal size. The protein was also analyzed chemically and by electron paramagnetic resonance spectrometry and the analysis demonstrated that one molecule of the enzyme contains atoms of manganese that numbers between 1.6 and 1.8. The enzyme contained no substantial amounts of copper or zinc (Keele *et al*., 1970).

Another difference in SODs from bacterial and mammalian sources is the detection of occurrence of reactive oxygen species by regulators that are distinctive from SoxR and OxyR. A good example of this is the regulation by PerR repressor in *Bacillus subtilis*. PerR is a metal-binding protein with Fur-like structure that suppresses the expression of catalase which is an alkyl hydroperoxidasereductase, and of Dps-like protein. It is proposed that PerP activity might be controlled by metal-catalyzed oxidation of a bound metal ion (Storz and Imlay, 1999).

Manganese superoxide dismutases

This enzyme, whether from bacteria or from the mitochondrial matrix, showed discernible sequence similarity, revealing a close evolutionary history and showing an endosymbiotic origin for mitochondria. There is some structural difference between bacterial and mitochondrial Mn SOD. The bacterial SOD is generally a homodimer, and the corresponding enzyme from mitochondria is a homotetramer. The molecular weight of subunit of this enzyme is 23 kDa. Some bacterial enzymes are also tetrameric as is the case of *Cryptococcus neoformans*. Providing the similarity in primary and three-dimensional structures of the manganese and iron SODs (MnSOD and FeSOD), it can be concluded that they have undoubtedly evolved from a common ancestor. Although they are structurally homologous, the Mn- and FeSODs have distinct functional roles. Only in exceptional cases, the endogenous Mn (or Fe) is substituted by Fe (or Mn) while retaining the catalytic activity. The Mn- and FeSODs are further distinguished in their distribution among bacterial species. Strict anaerobes contain one SOD that is FeSOD. Bacterial aerobes usually contain an MnSOD or both Mn- and FeSODs. (The MnSOD is also widely found in eukaryotes (Steinman, 1987).

In *E. coli*, the biosynthesis of Mn SOD is under the control of the soxRS regulon, which coordinately up-regulates the expression of a number of genes in response to O_2. The constitutively expressed SOX R protein is transcriptionally inactive in its reduced form. It can be oxidized by O_2 and then activates the expression of the SOX S protein, which in turn

activates all the genes in the regulon. Thus, MnSOD is not measurable in extracts of anaerobically grown *E. coli*, but exposure of cultures to aerobic conditions elicits production of MnSOD. Increasing production of O_2, by raising pO_2, or by adding compounds such as viologens, which can mediate enhanced production of O_2, increases the level of Mn SOD. It has been possible to force *E. coli* to produce Mn SOD to 7% of its soluble protein by aerobic exposure to the viologenparaquat. The nectar of tobacco flowers has been found to contain a stable Mn-protein named nectarin that appears to be anMn SOD (Fridovich, 2004).

Structure of manganese superoxide dismutase

Several crystal structures of SODs have been determined. The known SOD structures fall into two groups: the Cu/Zn SODs fold into a flattened eight-stranded Greek-key β-barrel, while the MnSODs and FeSODs fold into two-domain structures mainly composed of α-helices (Wuerges et al., 2004). Crystal structures have been reported of the MnSODs from Homo sapiens (Hsieh et al., 1998) and many bacteria, including *Escherichia coli* (Edwards et al., 1998), *Bacillus halodenitrificans* (Liao et al., 2002), *B. anthracis* (Boucher et al., 2005), *Porphyromonas gingivalis* (Sugio et al., 2000) and *Thermus thermophilus* (Ludwig et al., 1991). All MnSOD structures comprise two domains: an α-helical domain and an α/β-domain (Liu *et al*., 2007).

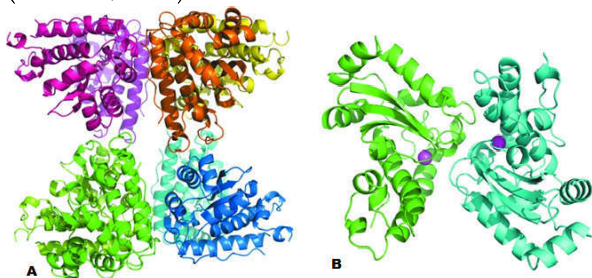


Fig. 3: Overall structure of B. subtilis MnSOD. (A) The asymmetric unit of the MnSOD structure is composed of four dimers, which are colored dark and light magenta, gold, blue and green. (B) The MnSOD dimer backbone colored by subunit with Mn atoms shown as magenta spheres (Liu et al., 2007).

Bacterial Mn/Fe SODS can be either homodimeric or tetrameric, whereas eukaryotic mitochondrial Mn SODS are tetrameric as are the cytosolic Fe SODS of plants and protists. The monomeric size of the Mn/Fe SODS is usually approximately 20-24 kDa. Mn/Fe SODS contain one metal ion per monomer. The metal is specific and replacement results in loss of activity, except for a few enzymes where Mn and Fe appear to be interchangeable. Mn/Fe SODS comprise mainly α-helices with the metal bound to three histidine residues and one aspartate (James, 1994). The combination of designed mutations and x-ray crystal structures were used to study metal site structure and function for both the cytoplasmic Cu, Zn superoxide dismutase (Cu, Zn SOD) and the mitochondrial Mn superoxide dismutase (MnSOD) (Tainer *et al*., 1993).

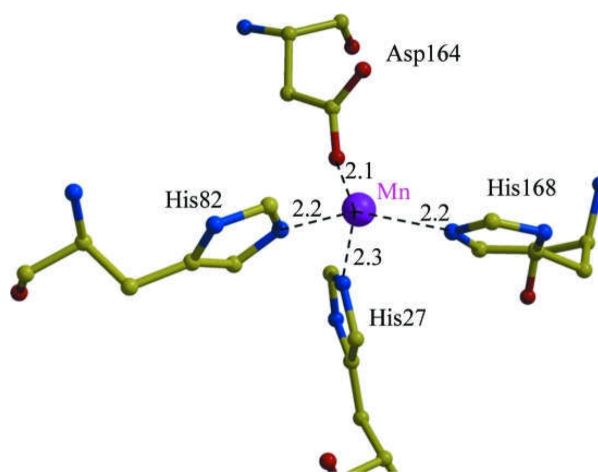


Fig. 4: Active site of MnSOD. Coordination of the Mn atom of B. subtilis MnSOD is shown. Interatomic interactions are shown as broken lines with distances in angstroms (Liu et al., 2007).

Mechanism of action of manganese superoxide dismutase

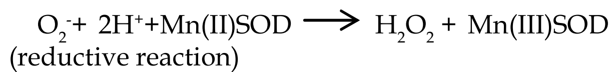
The primary function of MnSOD is to protect cells and mitochondria from free radical damage due to superoxide. Unlike the Cu/Zn- and Fe-containing enzymes, MnSOD expression in bacteria is induced under times of cellular stress as a result of exposure to a variety of elements including interleukin-1, tumor necrosis factor, paraquat, and X-ray radiation. The induction cascade is not fully understood: however, it is related to the superoxide response regulator (SoxR)-mediated pathway and correlates with both metal concentrations in the cell as well as the cell's redox environment, being activated when it is oxidative. In eukaryotes, MnSOD is targeted to the mitochondria after it is expressed in the nucleus. As over 90% of the dioxygen used by an organism is processed in the mitochondria, MnSOD primarily encounters reactive oxygen species formed as a result of mitochondrial function (Stroupe, 2011).

Superoxide dismutase (SOD) acts as the first line of defense against free radicals, it catalyzes the dismutation of superoxide anion radical ($O_2^{\bullet}$) into hydrogen peroxide (H_2O_2) by reduction. Enzyme undergoes both oxidation and reduction therefore the metal ion present at the centre be

both oxidizable and reductable by superoxide (Fee *et al,* 1973; Sawyer *et al.,* 1979). There is an electrostatic attraction between metal centre and superoxide anion.

The enzymatic reaction comprises of two distinct half reactions, an oxidative reaction in which the substrate, O_2^- is oxidized to dioxygen and a reductive half reaction in which O_2^- is converted into H_2O_2

O_2^- + Mn(III)SOD $\longrightarrow$ O_2 + Mn(II)SOD (oxidative reaction)



O_2^-+ $2H^+$+Mn(II)SOD $\longrightarrow$ H_2O_2 + Mn(III)SOD (reductive reaction)

The H_2O_2 formed is converted into water and oxygen (O_2) by catalase (CAT) or glutathione peroxidase (GPx). SOD converts superoxide to H_2O_2, a relatively stable molecule (Fig. 5). Although it occurs spontaneously, the role of SOD is to increase the rate of the reaction to that of a diffusion-controlled process. In the cytosol and the intermembrane space of mitochondria, superoxide is eliminated by Cu, Zn-SOD, whereas in the matrix, it is eliminated by Mn-SOD (Bayir and Kagan 2008).

Applications

Superoxide dismutases enzymes has great potential to act as an anti-aging agent, it has been demonstrated previously that with the increase in age the SOD level goes down, at the same time free radical levels increase. Another therapeutic application of Manganese superoxide dismutase (MnSOD) is that it can be used as a biomarker of different human diseases and this can help in the prevention of cancer and its treatment (Moradi *et al.,* 2015). A new form of human MnSOD is isolated from a human liposarcoma cell line (LSA) was able to kill cancer cells expressing estrogen receptors, but it did not have cytotoxic effects on normal cells (Borreilli *et al.,* 2014). Other applications of SODs in their topical forms are to help to reduce facial wrinkles, scar tissue, heal wounds and burns, lighten dark or hyperpigmentation, and protect against harmful UV rays.

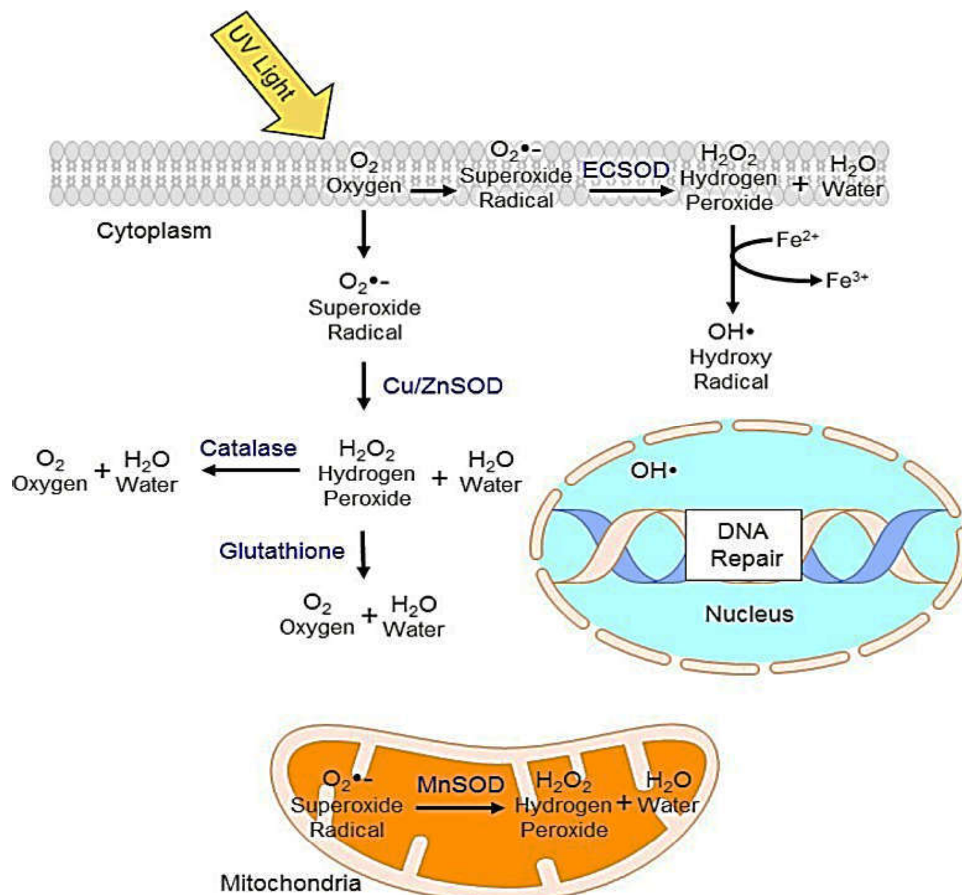


Fig. 5: Mechanism of action of SOD (Bayir and Kagan 2008)

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