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Review

The basic and applied aspects of superoxide dismutase

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ABSTRACT

Superoxide dismutase (SOD) is among the most potent antioxidants known in nature and is an important constituent of cellular defence against oxidative stress. The enzyme shows several interesting properties like very high catalytic rate of reaction and high stability to physico-chemical stress. It has also attracted widespread interest due to its therapeutic potential. Oxidative stress is known to be involved in pathophysiology of several diseases and SOD supplementation has been shown to be beneficial in treatment or prevention of such diseases. However, it is yet to be developed into effective, reliable and safe antioxidant therapy. Current review focuses on the physiological importance of SOD, and developments and obstacles in its therapeutic applications for treatment of various disorders. The review also summarizes SOD-based products and patents, its potentiation to improve efficacy, and major clinical trials of SOD in human subjects. Besides, latest literature on phylogeny and biochemistry of the enzyme is also reviewed.

Contents

1. Introduction

Although oxygen (O_2) is known to be essential for life, its toxic properties were also discovered long back. In as early as 1954, O_2 toxicity was suggested to be mediated through free radicals or, more generally, reactive oxygen species (ROS) [1]. ROS are highly reactive oxygen-containing products of normal cellular metabolism that can cause oxidative stress by damaging cellular macromolecules. Importance of ROS in biological systems was then established by a series of discoveries, which indicated that living systems have not only adapted to protect themselves from ROS, but have also evolved mechanisms for the advantageous use of ROS in various physiological functions [2]. A major defence mechanism against ROS was explored in 1969 when McCord and Fridovich reported dismutation of superoxide radical ($O_2^{\bullet}$) by erythrocuprein and suggested the name superoxide dismutase (SOD; EC 1.15.1.1) for it [3]. Erythrocuprein protein was discovered 30 years earlier by Mann and Keilin as a bovine liver protein of unknown function. SOD is found in almost all organisms exposed to oxygen and is responsible for converting $O_2^{\bullet}$ into hydrogen peroxide (H_2O_2) and O_2. H_2O_2 is further converted into harmless product water by other enzymes like catalase and peroxidases.

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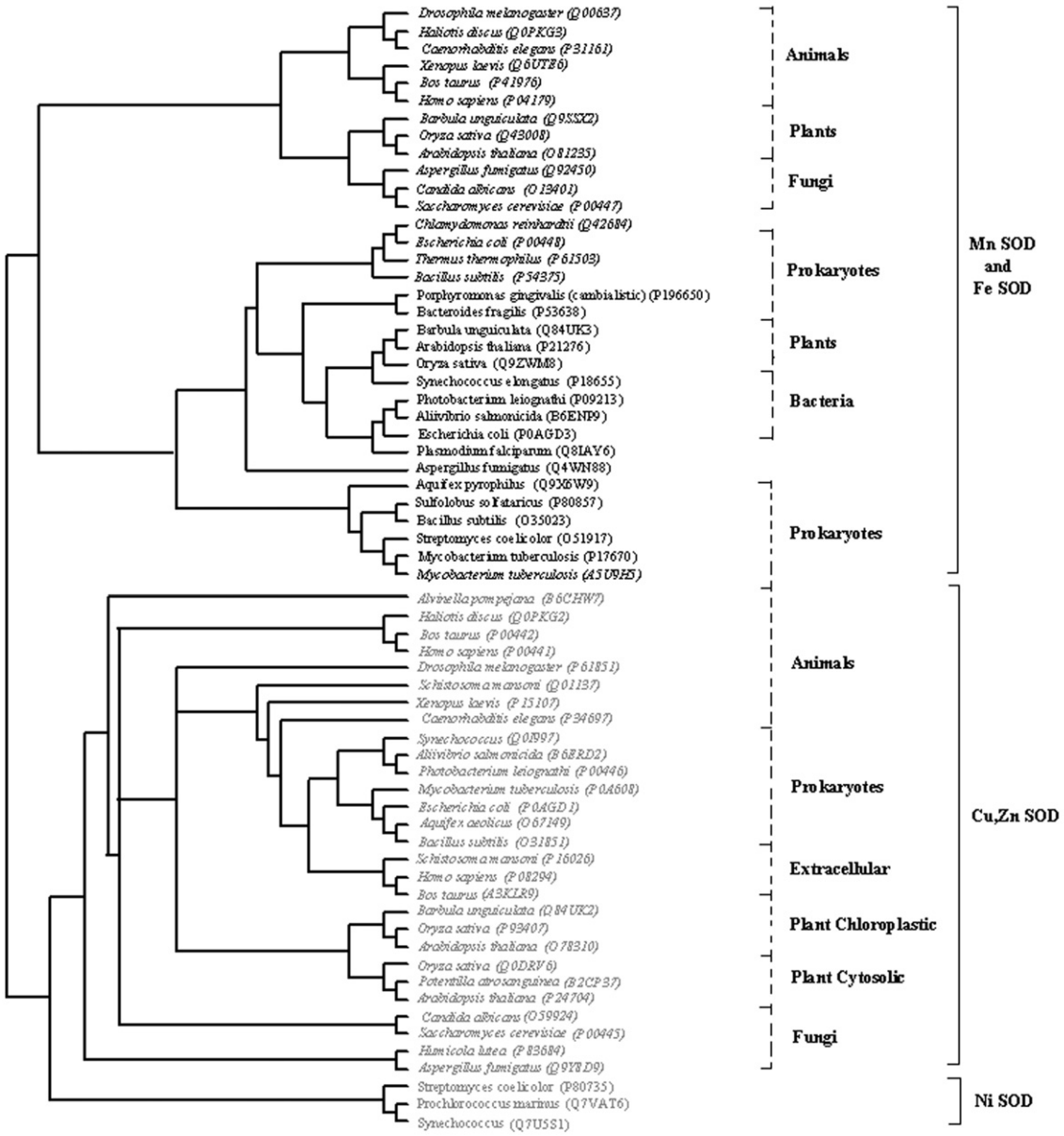

Fig. 1. Neighbour-joining phylogenetic tree based on the amino acid sequences of representative SOD enzymes. Different SOD classes have been colour-coded as: Cu,Zn SODs – grey italic, Mn SOD – black italic, Fe SOD – black plain, and Ni SOD – grey plain letters. SWISS-PROT IDs are given in parentheses after the names of source organisms.

2. Distribution and phylogeny

SOD is ubiquitous to all forms of life. Four different types of metal centers have been detected in SOD, dividing this family into Cu,Zn-, Fe-, Mn- and Ni-SODs. The evolution of SOD and other antioxidant enzymes was probably triggered by production of O_2 by photosynthetic organisms about 2 billion years ago. Two major kinds of SOD appeared independently in prokaryotes at that time, Cu,Zn SODs and Fe SODs/Mn SODs (Fe SOD probably being more ancient, see below). Fe/Mn SODs then evolved into Fe and Mn SODs by gene duplication (Fig. 1). This may be the reason why Fe and Mn SODs are closely related with regard to three-dimensional structure and amino acid sequence. However, their crystal structures and catalytic mechanism are completely different as compared to Cu,Zn SOD, supporting the hypothesis of independent evolution [4].

The Cu,Zn SOD is found mainly in eukaryotes, chloroplast and bacteria. In bacteria, it is encoded by the *sodC* gene and is located in the periplasm. Animals have two different forms of Cu,Zn SOD-cytosolic SOD1 and extracellular EC SOD/SOD3. SOD3 is distinct from SOD1 in terms of molecular weight as well as amino acid composition. The evolutionary tree for Cu,Zn SOD, based on multiple sequence alignment and structural superimpositions of crystal structures, shows that EC SOD diverged from SOD1 at an early stage of evolution, even before the differentiation of fungi, plants, and metazoa [5]. Unlike other organisms, plants have been reported to possess multiple forms of Cu,Zn SOD, which are encoded by more than one gene. Phylogenetically, they group with the eukaryotic Cu,Zn SODs, and are clustered into two main subgroups of chloroplastic and cytosolic SODs, indicating that these two subgroups diverged early in the evolution of plants [6].

Mn SOD occurs in prokaryotes and mitochondria of the eukaryotes. In prokaryotes it is encoded by the *sodA* gene, while in animal mitochondria it is known as SOD2. Similarly, Fe SOD is found in prokaryotes (SodB) and chloroplasts. The mitochondrial

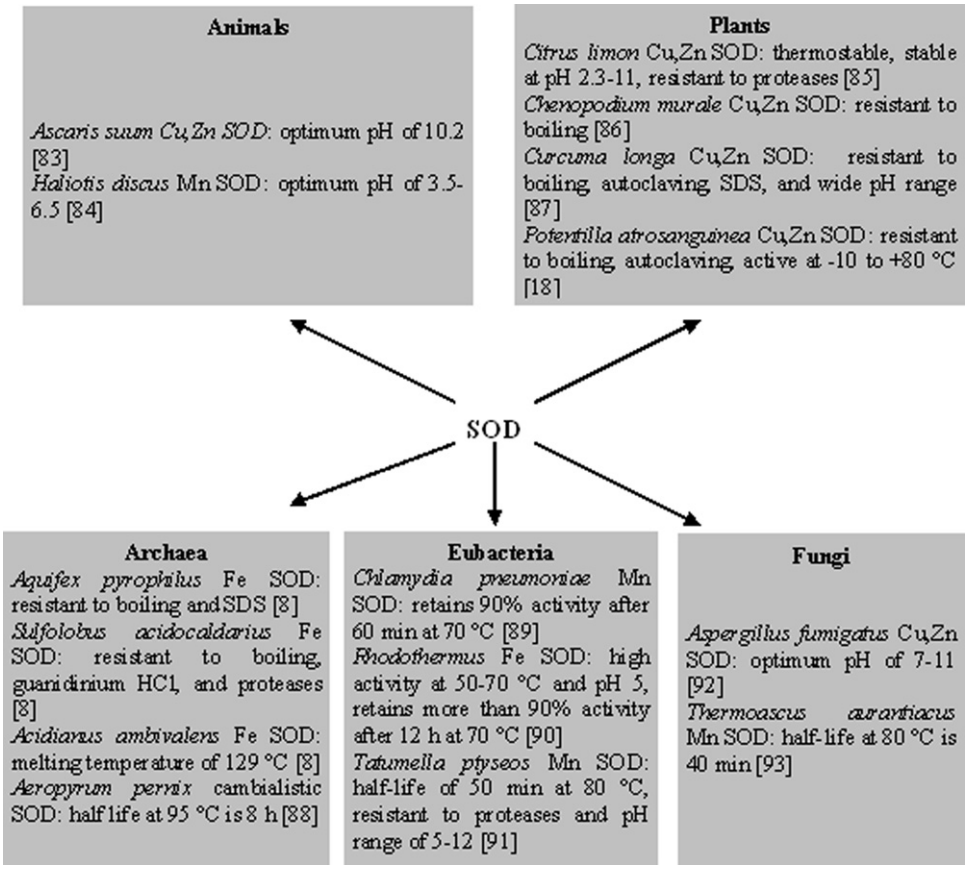

Fig. 2. Physico-chemical stability of SODs from certain sources.

and chloroplastic Mn- and Fe SODs have been proposed to be prokaryotic in origin. The sequences of tobacco and *Arabidopsis* chloroplastic Fe SODs have been shown to be most similar to that of cyanobacterium, *Anacystis nidulans* [7], and their origin probably lies with the endosymbiont. Apart from chloroplast and mitochondria, higher organisms also accumulated SODs in other compartments where O_2 could be activated (microsome, peroxisome, glyoxysome, extracellular matrix and cytosol). This was important because $O_2^{\bullet}$ is membrane-impermeable and must be detoxified in the same compartment where it is formed.

In the Fe- and Mn SOD group, Fe SOD is proposed to be more ancient because of an abundance of Fe in soluble Fe(II) form on primitive earth. As the level of O_2 in the primitive environment increased, availability of Fe(II) decreased, probably causing a shift to the use of more available Mn. A phylogenetic tree of Fe- and Mn SODs shows short distances separating Fe SODs from Mn SODs, confirming a common phylogenetic origin for these two, and suggesting likely frequent horizontal gene transfer. The tree also clearly separates archaeal SODs from other prokaryotic SODs [8]. Since archaea and eubacteria separated much before the appearance of O_2, this raises the question that SOD first appeared in which of these two lineages.

Many of the archaeal Fe- and Mn SODs are extremely thermostable. The high apparent T_m values of these enzymes (some about 40 °C above the optimal growth temperature) suggest their evolutionary origin under the conditions of the early earth, i.e. a hot environment. Surprisingly, these hyperthermophilic SODs display high activity over a broad range of temperatures, including room temperature. From biochemical point of view, the high T_m values coupled with a broad range of operating temperature imply that these enzymes exist in quasi-frozen state, unlikely to require any large-scale conformational change in the course of reaction cycle. Thus, the hyperthermophilic SODs could easily adapt to the moderate temperatures of mesophilic life style, and hence, SOD may be considered as an archaic protein [8]. As an evidence, the Fe- and Mn SODs from psychrophiles like *Pseudoalteromonas haloplanktis* and *Aliivibrio salmonicida* have been found to be heat stable at temperatures well above their maximum growth temperatures [9,10].

Ni-dependent enzymes (SodN) have only been described from *Streptomyces* and cyanobacteria so far and hence, not much information is available about them. Dupont et al. [11] analyzed putative Ni SOD sequences from public database and observed that most of them belonged to marine environment. Further, the analysis revealed that the putative sequences were quite divergent from previously characterized Ni SODs. The putative sequences formed four well-separated clusters, with evidence of horizontal gene transfer among diverse phylogenetic backgrounds, including both eukaryotes and prokaryotes.

3. Biochemical properties

The catalytic mechanism of SOD is described by the following reaction sequence:

$$M^{3+} + O_2^{\bullet} + H^+ \rightarrow M^{2+}(H^+) + O_2$$

$$M^{2+}(H^+) + H^+ + O_2^{\bullet} \rightarrow M^{3+} + H_2O_2$$

where, M stands for metallic cofactor.

This stepwise mechanism confers several advantages to the reaction thermodynamics. Firstly, potential electrostatic repulsion between two $O_2^{\bullet}$ anions is overcome by reacting with only one molecule at a time. Specific binding to negatively charged $O_2^{\bullet}$

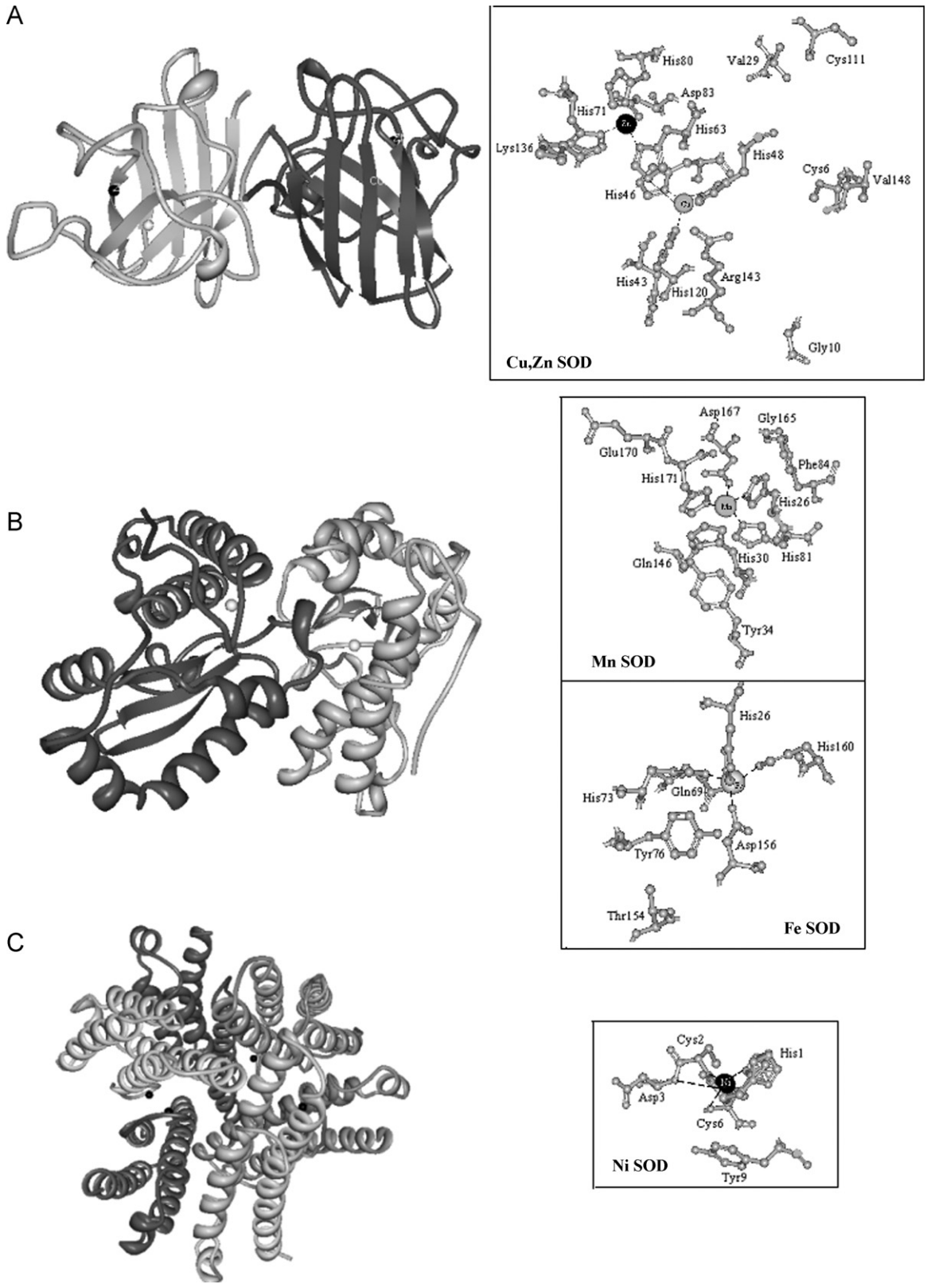

Fig. 3. Structures of different classes of SOD from the Protein Data Bank (PDB). (A) Human Cu,Zn SOD (PDB ID 2V0A), (B) *E. coli* Mn SOD (PDB ID 1D5N)/*E. coli* Fe SOD (PDB ID 1ISA), which show a similar structure, and (C) *S. coelicolor* Ni SOD (PDB ID 1T6U). The right panels show the active sites of above enzymes, including the hydrogen-bonding residues and other important residues mentioned in the text. Cu and Zn ions are depicted as white and black spheres respectively, Mn and Fe ions are depicted as white balls, and Ni ion is shown in black colour.

is mediated by the positively charged metals in the active site. In the second step with reduced metal ion, active site's electrostatic attraction is preserved by the uptake of a proton. The products of disproportionation are neutral and do not bind by this mechanism. Finally, the first half of the reaction is thermodynamically favourable and releases free energy, which is

utilized for the extremely unfavourable reduction of $O_2^{\bullet}$ in second step [12].

SOD exhibits several interesting properties. Firstly, the electron transfer mechanism between the substrate and the active site is considered to have reached perfection and the catalytic rate is only diffusion-limited. The reaction rate ($M^{-1}\,s^{-1}$) has been estimated to be about $6.4 \times l0^9$ for bovine erythrocyte Cu,Zn SOD, and 6.6×10^8 and 6.8×10^8 for *Escherichia coli* Fe- and Mn SODs respectively [13]. Secondly, the enzyme from several sources shows very high stability to urea, freeze–thaw cycles, high temperatures and unfavourable pH (Fig. 2).

Cu,Zn SODs are generally homodimeric (Fig. 3). Each monomer has a molecular weight of 14–33 kDa, and contains one Cu and one Zn atom. The catalytic activity of these enzymes is resistant to chemical (4% SDS, 8 M urea) and physical (heating, freezing, freeze–thaw cycles) treatments, and cleavage by proteinase K [14]. They are typically inhibited by azide, cyanide, diethyldithiocarbamate and H_2O_2. The EC Cu,Zn SODs from humans and other mammals are different in being homotetrameric. They also show some other differences from their cytoplasmic counterparts: (i) the central region of EC SOD is only about 50% identical to the final two-thirds of SOD1, although the amino acid residues involved in coordination of the Cu and Zn are conserved; (ii) antibodies derived against SOD1 do not react with EC SOD and vice versa; (iii) EC SOD has strong affinity for heparin present on cell surfaces, which is responsible for its extracellular localization. Removal of the heparin-binding domain of EC SOD through proteolysis is thought to control its mobility in tissues [15].

Fe- and Mn SOD types are typically homodimers or tetramers that contain one metal atom per subunit of 14–30 kDa (Fig. 3). Due to their structural and sequence conservation, they can frequently bind both Fe and Mn, but usually attain significant activity with only the permitted metal cofactor. This is thought to result from the tuning of the reduction potential provided by the active site environment. As an exception, a small group of these enzymes, termed cambialistic, are able to display significant activity with either of the two metals. Wintjens et al. [16] analyzed Fe- and Mn SOD sequences and crystal structures in the database to formulate indices differentiating between the two types. Resulting fingerprints allowed reliable prediction of metal cofactor, kinetic properties and stability of native as well as mutant enzymes, which were in good agreement with the available experimental results. Another important difference between the two enzymes lies in their sensitivity to different inhibitors. Fe SOD is inactivated by H_2O_2 and is resistant to KCN, while Mn SOD is resistant to both. Ni SODs have been found in very few organisms and generally function as homohexamers composed of ~13 kDa subunits [17].

4. Structure–activity relationship

Availability of crystal structures of SOD has greatly facilitated the determination of biochemical basis for its specificity, high activity and stability (Fig. 3). The authors' group has reported a highly stable Cu,Zn SOD from *Potentilla atrosanguinea* (Fig. 2). The general factors responsible for its stability were increase in the number of salt bridges and hydrogen bonds, and reduction in solvent-accessibility of hydrophobic residues. Comparison of its crystal structure with other SODs showed that it possessed Ile in place of Val147 at the dimer interface, and Ser in place of Gly10, which may contribute to its thermostability. Similarly, the enzyme was found to contain many Pro residues in or close to the loops connecting β strands of the backbone β barrel, a feature that has been suggested to enhance thermostability of enzymes. Further, it showed smaller gap between the two subunits, higher percentage of non-polar atoms, and lower fraction of solvent-exposed basic residues as compared to other Cu,Zn SODs [18]. Salt bridges and hydrogen bonds have also been proposed to be responsible for the biophysical stability of Cu,Zn SOD from the thermophilic annelid *Alvinella pompejana* [19].

Site specific mutants have been constructed to identify key amino acid residues involved in SOD activity and stability. Thermostability of Cu,Zn SODs has been correlated with free Cys residues in several organisms (Fig. 3). Human Cu,Zn SOD has a buried Cys6 located in a β strand, and a solvent accessible Cys111 located in a loop region. The homologous bovine enzyme has a single buried Cys6 residue. When these residues were replaced by Ala and Ser, the mutant human enzymes showed improved thermostability, while retaining their normal specific activity. This was found to be due to the stabilizing effects of side-chain to main-chain hydrogen bonds in one of the loops of β barrel [20]. $O_2^{\bullet}$ is normally steered to the active site of Cu,Zn SODs by a well-conserved electrostatic loop (containing positively charged residues like Lys136 and Arg143) near the active site. As SOD reacts with $O_2^{\bullet}$ at rates limited only by diffusion, it was proposed that its efficiency might be improved by electrostatic guidance, i.e. by enhancing the positive charge or accessibility at the active site. This was shown to be the case in *Photobacterium leiognathi* Cu,Zn SOD, whose catalytic rate increased by two times upon Val29 → Gly mutation at the subunit interface. It was found that the loops surrounding the active site were more flexible and the mobility of Trp83 was restrained in the mutant, making Cu more accessible to the incoming substrate. A Glu59 → Gln mutation also increased the reaction rate dramatically by improving the electric field and active site accessibility [21]. Similarly, site specific mutations that increased the local positive charge in human Cu,Zn SOD were found to impart faster reaction rates. Conversely, the activity was reduced significantly upon replacement of active site Arg143, indicating that it plays an important role in the catalytic process [22]. Cu,Zn SODs contain a conserved, metal-free His41 residue at the opening of the β barrel. His41-mediated hydrogen bond has been shown to play a crucial role in keeping the β-barrel structure stable for efficient catalysis [23].

Due to the high level of sequence and structural similarity between Fe- and Mn SODs, several mutagenesis studies were directed to identify the residues involved in metal ion specificity of the enzyme (Fig. 3). Gly165, which is located close to the active metal site, is mostly conserved in Mn SODs, but is substituted for Thr in most Fe SODs. A Gly165 → Thr mutation in *E. coli* Mn SOD led to appearance of catalytic activity in Fe-substituted enzyme [24]. It is observed that Gln69 in Fe SODs is complementarily substituted with Gln146 in Mn SODs, with the same orientation of the side chain amide group. The amide group is part of a hydrogen bond network that includes the metal–ligand solvent. A *Porphyromonas gingivalis* cambialistic SOD with mutations in the corresponding residues (Gln70 → Gly, Ala142 → Gln) showed an increase in Mn specificity of the enzymatic reaction. A similar result was reported on mutating corresponding residues of *E. coli* Mn SOD. The enzymatic activity of Fe-substituted Gly77 → Gln, Gln146 → Ala mutant enzyme was 7% of that of Fe SOD, in contrast to the Fe-substituted wild-type Mn SOD, which had no activity. Another difference is that Tyr77 in Fe SODs is changed to Phe in Mn SODs [25].

A unique characteristic of Mn SOD is the formation of a product-inhibited complex. The magnitude of inhibition varies among Mn SODs from different sources, appearing more prominent in human as compared to bacteria. Several reports have shown that a hydrogen-bonded network, comprising residues like His30, Tyr34, Gln143 and Tyr166, at the active site contributes to product inhibition. Certain mutations in these residues in human Mn SOD (Tyr34 → Phe) produced a significant increase in the rate of product inhibition, while other mutations (His30 → Asn/Ser, Gln143 → Asn/Ala/Gly/His/Asp/Glu) showed significantly reduced product inhibition [26]. The reduction in product inhibition, how-

ever, was achieved at the cost of reduced catalytic efficiency. When Gln143 → Ala mutation was complemented by other substitutions (Asn73 → Ser/Cys140 → Ser), catalytic activity was restored while maintaining the low product inhibition [27]. These complementary substitutions changed the hydrogen bonding network and reoriented the active site, resulting in increase in catalytic efficiency. Mutations in the vicinity of active site Tyr34 residue (Phe66 → Ala/Leu mutation at the dimeric interface of human Mn SOD) have also been found to reduce the level of product inhibition, indicating contribution of the active site environment. Ile58 residue located at the subunit interface has been shown to be involved in both activity and thermostability in human Mn SOD [28]. A hydrogen bond between Glu162 and His163 is also involved in enzyme activity and thermostability [29].

In Fe SOD Gln69 can strongly influence redox activity of the metal center due to its hydrogen bonding with metal–ligand solvent. Gln69 → His mutation in *E. coli* Fe SOD was found to preserve 30% activity, while mutation to Glu inactivated the enzyme completely. Redox titrations indicated that mutation to His increased the reduction potential by 240 mV, whereas mutation to Glu increased it by more than 660 mV [30]. Very high stability of Fe SODs from thermophilic archaea has attracted several studies. A structural comparison of extremely stable Fe SODs from *Sulfolobus acidocaldarius*, *Sulfolobus solfataricus* and *Aquifex pyrophilus* with slightly less stable Mn SOD from *Thermus thermophilus*, and mesophilic Mn SOD from *Mycobacterium tuberculosis* revealed the determinants which are probably responsible for the high intrinsic stability. The most obvious factors were increase in inter-subunit ion pairs and hydrogen bonds, significant reduction of solvent-accessible hydrophobic surfaces, and increase in the percentage of buried hydrophobic residues [8]. Moreover, average hydrophobicity and amino acid mean weight were found to directly correlate with thermostability in these enzymes. Mutational analysis of *A. pyrophilus* Fe SOD indicated that Lys12 → Ala mutation increased its thermostability, while Glu41 → Ala mutation reduced the thermostability. Both the mutations resulted in the replacement of ion pair network with hydrogen bonds. These results show differential contribution of ion pair networks in thermostability of proteins [31]. Tyr41 and His155 residues in *S. solfataricus* Fe SOD have been shown to be important for its structural and functional properties. In particular, Tyr41 seems to be a powerful regulator of the activity, whereas His155 is involved in organization of the active site [32].

Only few mutagenesis studies have been carried out on Ni SOD, most of them in *Streptomyces coelicolor* (Fig. 3). His1 and Tyr9 residues are involved in proton donation during Ni SOD catalysis. Bryngelson et al. [33] showed that His1 mutation significantly reduced its catalytic activity. Similarly, Tyr9 mutation was found to result in a saturation behaviour, indicating that it is involved in substrate access. Asp3 mutation could also bring about similar effect by repositioning Tyr9 [34]. Two Cys residues (Cys2 and Cys6) are coordinated to the metal center in Ni SODs. Mutations in these residues (Cys2/Cys6 → Ser) can completely inactivate the enzyme, indicating their role in stabilizing the metal center [35].

5. Physiological importance in humans and other animals

The ROS-induced oxidative stress has been implicated in pathophysiology of several conditions, such as aging, infertility, cardiovascular diseases, atherosclerosis, neurological disorders (Alzheimer's disease and Parkinson's disease), ischemia–reperfusion injury, transplant rejection, autoimmune diseases, rheumatoid arthritis, diabetes, asthma, septic shock-induced tissue injury, and cancer [36]. This is not only due to the ability of ROS to damage the cells directly but also due to their involvement in cell signaling, which regulates key processes like inflammatory, apoptotic and proliferation pathways. Oxidative stress may be caused by several reasons, such as ROS secretion by inflammatory phagocytes, hyperoxia, ROS activation by exogenous agents, increased ROS generation by mitochondria, reduced repair of oxidatively damaged proteins and DNA, decreased degradation of oxidatively damaged proteins by the proteasome, and reduced antioxidant capacity [37]. Indeed, reduced antioxidant capacity has been implicated in several incidences of the above conditions, and antioxidant supplementation has been shown to prevent or reverse the adverse effects. Antioxidants exert their effect not only by scavenging deleterious free radicals, but also by normalization of the ROS-mediated cell signaling. This is helpful in (i) protection of healthy cells from oxidative damage; (ii) preservation of normal cellular regulation; (iii) inhibition of proliferation and induction of apoptosis in cancer cells; (iv) inhibition of tumour invasion and angiogenesis; (v) preservation of Fe homeostasis; (vi) suppression of inflammation and autoimmune conditions.

Numerous papers have been published showing reduced levels of antioxidant enzymes in cancer cells. Cancer cells are nearly always low in Mn SOD and catalase activity, and usually low in Cu,Zn SOD activity. Normalization of SOD level has been shown to result in reversal of at least part of the cancer cell phenotype [38]. SOD activity has also been shown to decrease with age, which results in increase in the level of ROS, indicating that ROS may be involved in aging process. Cutler [39] showed that mammals that produce higher tissue and serum levels of SOD live longer than those with lower SOD levels. SOD levels among humans may vary by as much as 50%, which may explain why some people are more prone to degenerative disorders while others lead long, disease-free lives. These observations encouraged researchers to study the effect of SOD augmentation on longevity in different organisms. Augmentation of SOD level increased life span of invertebrates like *Caenorhabditis elegans* and *Drosophila melanogaster* in some of the studies [40], but showed no effect in other studies [41]. Also, no study has demonstrated successful application of SOD for increase in life span in mammals. Thus, the results are contradictory, though majority of the studies observed that augmentation of SOD activity reduced oxidative stress while its deletion led to increase in oxidative stress. This has encouraged many researchers to question the basic oxidative stress theory of aging.

Amyotrophic lateral sclerosis (ALS), a selective, neurodegenerative disease, is known to be caused by a dominant mutation in the *sod1* gene, although the pathophysiological mechanism is still controversial. Many other diseases have been directly linked to the deficiency of SOD. SOD deficiency is proposed to correlate with conditions like infertility in men [42] and mental disturbances resulting in self-aggressive behaviour [43]. Increased level of Cu,Zn SOD, on other hand, is shown to correlate with sepsis and risk of mortality in patients [44]. Similarly, polymorphism studies have shown certain SOD genotypes to be linked with conditions like noise-induced hearing loss, essential hypertension, vasospastic angina pectoris, increased risk of hepatocellular carcinoma in alcoholic cirrhotic patients, and death from breast cancer [45–47]. SOD-related disorders need not always be caused by genetic defects. In a study by Reeves et al. [48] male rats fed with a Cu-deficient diet showed decreased SOD1 and SOD3 activity. Diet has also been shown to regulate SOD level epigenetically. Thaler et al. [49] showed that vegetarians show decreased CpG methylation in promoter resulting in increased Mn SOD expression as compared to omnivores group. Similarly, alcoholism and cigarette smoking lead to oxidative stress and reduced SOD level.

Mn SOD has been shown to be essential for life, while the other two Cu,Zn SODs are dispensable. Lebovitz et al. [50] demonstrated that deletion of the Mn SOD gene in mice resulted in death within 5–21 days of birth. Similarly, Mn SOD (−/+) heterozygous mice (expressing 50% of the normal complement of Mn SOD) showed

oxidative damage and functional alterations in mitochondria. Paul et al. [51] reported shortened life span with reduction in Mn SOD expression in *Drosophila*. The importance of Mn SOD can be attributed to the fact that more than 90% of O_2 is consumed in the body by electron transport chain of mitochondria, and about 1–5% of it is released as $O_2^{\bullet}$ and H_2O_2. Because of the high level of internally generated ROS, lack of histone protection, and a low level of DNA repair, mitochondrial DNA is particularly vulnerable to oxidative damage. Mn SOD functions to preserve mitochondrial integrity by scavenging $O_2^{\bullet}$. Interestingly, Mn SOD itself is highly prone to oxidative inactivation. While human Cu,Zn SOD contains no Tyr residues and appears to be very resistant to ROS, human Mn SOD contains nine Tyr residues, some of which can be easily oxidized. Thus, changes in Mn SOD activity need not depend on the rate of protein synthesis or degradation, and can occur very quickly under pathological conditions [52].

6. Role in plants

In plants ROS play important role in signaling of pathways that respond to biotic and abiotic stress. ROS are also activated as defence molecules when plants are attacked by pathogens, or even symbionts like VAM fungi. Since ROS can indiscriminately damage both, the symbiont as well as the host cells, role of SOD is very important in protecting both the symbiotic partners [53]. Increased level of SOD can also protect plants against cold stress at high altitude and O_3 injury [54]. Introduction of SOD transgene into plants has been shown to produce desired phenotypes such as increased resistance to physical (chilling, drought, salinity and high light intensity) and chemical (O_3, metal ions, $O_2^{\bullet}$-generating herbicides) stress, and improved biomass production with larger shoot, crown and root systems [55]. SOD-mediated salt tolerance has been proposed to be imparted through lignification of vascular structures, which is induced due to increased H_2O_2 production by SOD [56]. Conversely, antisense suppression of Mn SOD led to reduced root growth, and affected tricarboxylic acid cycle flux and mitochondrial redox homeostasis [57].

Marine algae secrete ROS as signaling molecules for quorum sensing, or to reduce competition by killing bacteria and reducing Fe availability. The algae protect themselves from these biological ROS, and the photochemical ROS resulting from high light intensity, by producing high levels of SOD [58]. Similarly, Fe SOD has been shown to be essential for protection against chilling stress-mediated increase in ROS in the cyanobacterium *Synechococcus* [59].

7. Role in microorganisms

SOD confers protection against ROS-induced oxidative stress not only in higher organisms, but also in microbes. Since aerobic microorganisms are more prone to oxidative stress, SOD is present in O_2-metabolizing microbes, but is absent from many of the strict anaerobes. SOD1 mutation in the mycorrhizal fungus *Oidiodendron maius* resulted in an increased sensitivity to ROS-generating substances, and reduction in conidiation and mycorrhization [53]. In bacteria, Fe- and Mn SODs afford protection against O_2-dependent DNA damage due to their cytoplasmic location. Mutations in these genes increase the paraquat (generator of cytosolic $O_2^{\bullet}$) sensitivity, decrease survival in stationary phase, and produce a variety of O_2-dependent phenotypic alterations including severe defects in amino acid biosynthesis, structural instability of the cell envelope, and a high rate of spontaneous mutagenesis [60].

The periplasmic Cu,Zn SOD in bacteria is likely to protect periplasmic proteins against endogenous $O_2^{\bullet}$. It is induced maximally in aerobic stationary phase and is required for survival in this phase [61]. In pathogens, periplasmic Cu,Zn SOD performs a more significant role by scavenging extracellular ROS derived from the oxidative burst of phagocytes. Overexpression of SOD, antibody blocking, and addition of exogenous SOD have demonstrated its protective role in pathogens against the host cells [62]. This is true not only for bacterial pathogens, but also for eukaryotic pathogens.

8. Therapeutic applications

From the above discussion, it is clear that SOD is a promising candidate for the treatment of a wide variety of oxidative stress-mediated diseases. Hence, it has been a focus of immense research in animal and in vitro models using following approaches: (i) the native SOD activity and/or gene expression can be increased. Overexpression of SOD protects cells against pro-apoptotic stimuli and ischemic damage, as demonstrated by prevention of optic neuropathy induced by deficiency of mitochondrial complex I, prevention of alcohol-induced liver injury, improvement of erectile function in aged rats, and prevention of HIV tat-induced neuronal apoptosis [63–65]. Overexpression of SOD has also been used to inhibit cancer cells through H_2O_2-mediated toxicity, where removal of H_2O_2 is prevented by using enzyme inhibitors [66]; (ii) SOD mimetics such as M40403 (designated as orphan drug by US Food and Drug Administration for the prevention of radiation/chemotherapy-induced side effects in cancer patients), porphyrin-based compounds, or salen-manganese compounds have been shown to have protective effects in several conditions [67]; (iii) supplementary SOD therapy has been shown to prevent conditions like clastogenesis and cell death in trauma and ischemia, hyperoxia-induced pulmonary injury, emphysema, tissue injury in infections like bacterial meningitis and viral influenza, ROS-mediated damage induced by certain therapeutic drugs, and cytotoxicity caused by oxidative stress-inducing pollutants like PCBs [68–70]. SOD supplementation can also be used for resolution of inflammation. Exogenously added SOD cannot only scavenge inflammatory ROS, but also activate neutrophil apoptosis through H_2O_2-induced caspase pathway. SOD has shown promising results in the treatment of several inflammatory conditions like osteoarthritis, dental pulp inflammation, ischemia–reperfusion injury, and liposome-mediated inflammation during liposomal drug delivery [71].

Several clinical trials have been undertaken in human subjects to demonstrate therapeutic potential of SOD. However, results are of mixed type. While some studies indicated promising results (Table 1), neutral or even negative outcomes were obtained in other studies (Table 2). Most of these studies used human or bovine Cu,Zn SOD as the intervention agent. Since SOD catalyzes production of H_2O_2, which is also a powerful oxidant, some of the studies used SOD in combination with other antioxidants like catalase. Inspired from the positive findings, several leading biotechnology and pharmaceutical companies filed a number of patents for commercial development of SOD-based products (Table 3). However, none of these products could enter the market due to inconsistent outcome of clinical studies. An orally effective form of SOD (glisodin) was developed by Isocell Pharma by combining Extramel (cantaloupe melon extract which is rich in SOD) with wheat gliadin. Glisodin showed some cosmetic and health benefits in human subjects (Table 4), but it is still not approved for therapeutic use. The only SOD product approved for therapeutic application is orgotein from Oxis International Inc., although it is approved for animal use only and not human application [72].

9. Bioengineering to enhance the efficiency of SOD

As discussed in above section, there are significant advances towards therapeutic application of SOD. However, in many of the

Table 1
Clinical studies of SOD in human subjects with promising results.

No.	Study	Inferences	References
1	Treatment of chronic cystitis, chronic prostatitis and hydrocele	Effective	[94]
2	Treatment of epicondylitis	Effective	[95]
3	Treatment of rheumatoid arthritis and osteoarthritis	More/equally effective to aspirin and steroids like methylprednisolone acetate	[96,97]
4	Treatment of progressive systemic sclerosis, systemic lupus erythematosus, Behçet's disease, herpes simplex and burns with topical and injectible SOD	Effective	[98,99]
5	Treatment of skin inflammation, burn and wounds with topical and injectable SOD	Effective, inhibited lipid peroxidation and damage to visceral organs	[100,101]
6	Treatment of Crohn's disease	Successful	[102]
7	Prevention of multiple organ failure after multiple trauma	Attenuation of organ failure	[103]
8	Prevention of ischemia–reperfusion injury	Improved graft survival	[104,105]
9	Anti-radiotherapy effect of free, liposomal or PEGylated SOD	Reduction in fibrosis and adverse reactions	[106]
10	Treatment of temporomandibular joint dysfunction	Effective in 83% cases	[107]
11	Treatment of respiratory distress syndrome in premature neonates	Some benefits	[108]
12	Safety and clinical trials of lecithinized SOD	Effective in ulcerative colitis and noninfectious corneal ulcers	[109,110]
13	Treatment of Peyronie's disease with liposomal SOD	Beneficial	[111]
14	Vitiligo treatment with topical catalase/SOD	As efficient as the betamethasone treatment	[112]

Table 2
Clinical studies of SOD in human subjects with discouraging results.

No.	Study	Inferences	References
1	Treatment of Duchenne's muscular dystrophy	No effect	[113]
2	Prevention of radiation-induced side effects	No benefits, caused allergic reactions	[114]
3	Prevention of ischemia–reperfusion injury	No effect	[115,116]
4	Treatment of essential hypertension	No effect	[117]
5	Treatment of severe closed head injury with pegorgotein	No effect	[118]
6	Treatment of ALS by intrathecal administration of SOD	No effect	[119]
7	Pegorgotein application to patients undergoing coronary bypass surgery	No effect	[120]
8	Vitiligo treatment with topical catalase/SOD	No effect	[121]

Table 3
International patents for commercialization of SOD.

No.	Company	Product	Patents
1	Bio-Technology General Corp. (now Ferring International, Switzerland)	Oxsodrol	US patents 5360729, 5670371
2	Chiron (now Novartis, Switzerland)	Recombinant human Cu,Zn SOD	US patents 5084390, 5252476, 5691139, 6326003; PCT application WO 001503
3	Estee Lauder, US	Cosmetic application of SOD	US patents 4786493, 4839164
4	Isocell Pharma, France	Orally effective from of SOD (glisodin) using extramel and wheat gliadin	US patents 6045809, 6426068; PCT application WO 105024
5	L'Oreal, France	Cosmetic application of SOD	US Patents 4129644, 5352438, 5650137, 5925363; PCT application WO 019224
6	Millennium Biotechnologies, US	Glisodin formulations, resurgex and resurgex plus	US patent 6503506
7	Oxis International Inc., US	Orgotein	US patents 3579495, 3637640, 3773929
8	Pentapharm, Switzerland	Recombinant SOD from baker's yeast (dismutin-bt)	US patents 4695456, 4957740, 6451559
9	Seppic, France	Extramel (SOD-enriched extract of cantaloupe melon)	US patents 5616323, 7132118

Table 4
Clinical studies of glisodin (Isocell Pharma, France) in human subjects.

No.	Study	Inferences	References
1	Prevention of hyperbaric oxygen-mediated damage	Effective in reducing DNA damage and lipid peroxidation	[122]
2	Controlling atherosclerosis in subjects with risk factors of cardiovascular disease (genetic/familial factors, blood chemistry, blood pressure, body mass index, and cigarette smoking)	Effective	[123]
3	Effect of extramel (active ingredient of Glisodin) on stress and fatigue	Effective	[124]

clinical trials, application of SOD led to neutral or even negative outcomes, indicating the requirement for further improvements. The main issues to be addressed include its antigenicity, stability, pharmacokinetics, efficient targeting, and sustained release. The molecular weight of SOD is well below the renal glomerular filtration cutoff, resulting in a rapid clearance of exogenous SOD from the circulation. Hepatic uptake is another pathway responsible for its elimination. Several human trials have shown its half life to range from a few minutes to a few hours even at high doses [73]. Other obstacles include its ability to equilibrate between extracellular fluid compartments, approach negatively charged cell surfaces, and enter inside the cells. Several approaches have been found to reduce these problems:

(i) Entrapment of the enzyme into liposomes or transferosomes (ultradeformable lipid vesicles for non-invasive administration), and lecithinization. SOD administered transdermally in the form of transferosomes gave improved bioavailability and ameliorated the disease symptoms of arthritis in a rat model [74].
(ii) Masking of the protein surface by polymers (for example, polysaccharides like carboxymethylchitin, acrylic polymers, albumin, casein, succinylated keratin fragment, and polyethylene glycol/PEG) restricts the renal clearance by increasing molecular size [75].
(iii) Polymerization by linking SOD subunits with human immunoglobulin IgA1 hinge sequence [76].
(iv) Cellular SODs hardly bind to extracellular matrix and hence, are rapidly cleared. Cationization can greatly solve this problem [77]. SOD3 also seems to be an attractive option, but it binds with endothelium so tightly that its active soluble concentration is very low. A SOD2/3 chimera containing the mature human SOD2 fused to the heparin-binding domain of human SOD3 exhibited much improved pharmacological properties. The molecule bound to the endothelial cells less tightly and circulated well enough to become widely distributed. It was shown to be protective in a variety of inflammation and ischemia–reperfusion models [78].
(v) Binding to chemicals, such as antibodies, lectins, sugars, poly(vinylpyrrolidone-co-dimethyl maleic anhydride) co-polymer, tetanus toxin fragment, and heparin-binding peptides, for targeting to specific sites [75].
(vi) Since SOD cannot cross cell membrane, its intracellular delivery has been effected by approaches like fusion with cell penetrating peptides, coupling with polyketal microparticles, liposomal delivery, adeno-associated virus (AAV) vector-mediated delivery, ghost erythrocyte encapsulation, and chitosan microencapsulation [79]. Intracellular delivery in immune effector cells has been achieved by Fc receptor-mediated endocytosis of SOD immune complexes [80].
(vii) Apart from parenteral administration, oral and respiratory routes have also been tried and found to be promising. Intratracheally administered SOD has been found to get rapidly incorporated into lung cells and exhibit improved pharmacokinetics [81]. Since oral supplements can be destroyed by gastric acidity, they are protected by suitable coating or complexing with other proteins.
(viii) The clinical research work has only focused on human and bovine SOD. However, SODs from different sources vary widely in their stability and possibly pharmacokinetics too (Fig. 2). This encouraged few researchers to test SODs from alternative sources. Ratcheva et al. [82] showed that yeast SOD can protect mice against adjuvant arthritis without eliciting detectable antigenic response.

10. Conclusions

SOD is a phylogenetically heterogeneous group of enzymes. There are four different forms of SOD, namely Cu,Zn-, Fe-, Mn- and Ni SODs, widespread in all forms of life. SOD exhibits a very high catalytic rate of reaction. The electron transfer mechanism between the substrate and the enzyme is considered to have reached perfection, and the reaction is only diffusion-limited. SODs from various sources show very high stability to physico-chemical stress, such as urea, freeze–thaw cycles, high temperatures and prolonged refrigeration. Significant advances have been made in understanding the biochemical basis of such stability.

Increasing understanding of the role of free radicals in diseases is opening a new area for application of antioxidants in prevention and therapy of oxidative stress-related diseases. A number of epidemiological studies have suggested strongly that SOD can decrease the incidence of diseases. However, organized animal and human studies have yielded discouraging results. This can be attributed to problems like unfavourable pharmacokinetic profiles and inadequate delivery of SOD. SOD is poorly absorbed and rapidly degraded in the gastrointestinal tract, and has extremely short life span in the blood stream after intravenous administration. Hence, further formulation, pharmacokinetic and toxicological studies are still required to improve the delivery and safety aspects of SOD.

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References

[1] R. Gerschman, D.L. Gilbert, S.W. Nye, P. Dwyer, W.O. Fenn, Science 119 (1954) 623–626.
[2] M. Valko, D. Leibfritz, J. Moncol, M.T. Cronin, M. Mazur, J. Telser, Int. J. Biochem. Cell Biol. 39 (2007) 44–84.
[3] J.A. Imlay, Antioxid. Redox Signal. (in press).
[4] M.W. Smith, R.F. Doolittle, J. Mol. Evol. 34 (1992) 175–184.
[5] I.N. Zelko, T.J. Mariani, R.J. Folz, Free Radic. Biol. Med. 33 (2002) 337–349.
[6] R.C. Fink, J.G. Scandalios, Arch. Biochem. Biophys. 399 (2002) 19–36.
[7] W. Van Camp, C. Bowler, R. Villarroel, E.W. Tsang, M. Van Montagu, D. Inze, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 9903–9907.
[8] G. Schafer, S. Kardinahl, Biochem. Soc. Trans. 31 (2003) 1330–1334.
[9] I. Castellano, A. Di Maro, M.R. Ruocco, A. Chambery, A. Parente, M.T. Di Martino, G. Parlato, M. Masullo, E. De Vendittis, Biochimie 88 (2006) 1377–1389.
[10] H.L. Pedersen, N.P. Willassen, I. Leiros, Acta Crystallogr. Sect. F: Struct. Biol. Cryst. Commun. 65 (2009) 84–92.
[11] C.L. Dupont, K. Neupane, J. Shearer, B. Palenik, Environ. Microbiol. 10 (2008) 1831–1843.
[12] A.F. Miller, Curr. Opin. Chem. Biol. 8 (2004) 162–168.
[13] B. Gray, A.J. Carmichael, Biochem. J. 281 (1992) 795–802.
[14] J.V. Bannister, W.H. Bannister, G. Rotilio, CRC Crit. Rev. Biochem. 22 (1987) 111–180.
[15] C.L. Fattman, L.M. Schaefer, T.D. Oury, Free Radic. Biol. Med. 35 (2003) 236–256.
[16] R. Wintjens, D. Gilis, M. Rooman, Proteins 70 (2008) 1564–1577.
[17] D.P. Barondeau, C.J. Kassmann, C.K. Bruns, J.A. Tainer, E.D. Getzoff, Biochemistry 43 (2004) 8038–8047.
[18] M. Yogavel, P.C. Mishra, J. Gill, P.K. Bhardwaj, S. Dutt, S. Kumar, P.S. Ahuja, A. Sharma, Acta Crystallogr. D: Biol. Crystallogr. D64 (2008) 892–901.
[19] D.S. Shin, M. Didonato, D.P. Barondeau, G.L. Hura, C. Hitomi, J.A. Berglund, E.D. Getzoff, S.C. Cary, J.A. Tainer, J. Mol. Biol. 385 (2009) 1534–1555.
[20] H.E. Parge, R.A. Hallewell, J.A. Tainer, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 6109–6113.
[21] M. Falconi, M.E. Stroppolo, P. Cioni, G. Strambini, A. Sergi, M. Ferrario, A. Desideri, Biophys. J. 80 (2001) 2556–2567.
[22] W.F. Beyer Jr., I. Fridovich, G.T. Mullenbach, R. Hallewell, J. Biol. Chem. 262 (1987) 11182–11187.
[23] A. Toyama, Y. Takahashi, H. Takeuchi, Biochemistry 43 (2004) 4670–4679.
[24] M. Osawa, F. Yamakura, M. Mihara, Y. Okubo, K. Yamada, B.Y. Hiraoka, Biochim. Biophys. Acta 1804 (2010) 1775–1779.
[25] F. Yamakura, S. Sugio, B.Y. Hiraoka, D. Ohmori, T. Yokota, Biochemistry 42 (2003) 10790–10799.

[26] J.J. Perry, A.S. Hearn, D.E. Cabelli, H.S. Nick, J.A. Tainer, D.N. Silverman, Biochemistry 48 (2009) 3417–3424.
[27] K. Chockalingam, J. Luba, H.S. Nick, D.N. Silverman, H. Zhao, FEBS J. 273 (2006) 4853–4861.
[28] G.E. Borgstahl, H.E. Parge, M.J. Hickey, W.F. Beyer Jr., R.A. Hallewell, J.A. Tainer, Cell 71 (1992) 107–118.
[29] P.S. Quint, J.F. Domsic, D.E. Cabelli, R. McKenna, D.N. Silverman, Biochemistry 47 (2008) 4621–4628.
[30] E. Yikilmaz, D.W. Rodgers, A.F. Miller, Biochemistry 45 (2006) 1151–1161.
[31] J.H. Lim, K.Y. Hwang, J. Choi, D.Y. Lee, B.Y. Ahn, Y. Cho, K.S. Kim, Y.S. Han, Biochem. Biophys. Res. Commun. 288 (2001) 263–268.
[32] M.A. Gogliettino, F. Tanfani, A. Scire, T. Ursby, B.S. Adinolfi, T. Cacciamani, E. De Vendittis, Biochemistry 43 (2004) 2199–2208.
[33] P.A. Bryngelson, S.E. Arobo, J.L. Pinkham, D.E. Cabelli, M.J. Maroney, J. Am. Chem. Soc. 126 (2004) 460–461.
[34] R.W. Herbst, A. Guce, P.A. Bryngelson, K.A. Higgins, K.C. Ryan, D.E. Cabelli, S.C. Garman, M.J. Maroney, Biochemistry 48 (2009) 3354–3369.
[35] K.C. Ryan, O.E. Johnson, D.E. Cabelli, T.C. Brunold, M.J. Maroney, J. Biol. Inorg. Chem. 15 (2010) 795–807.
[36] A. Valdivia, S. Perez-Alvarez, J.D. Aroca-Aguilar, I. Ikuta, J. Jordan, J. Physiol. Biochem. 65 (2009) 195–208.
[37] I. Martin, M.S. Grotewiel, Mech. Ageing Dev. 127 (2006) 411–423.
[38] A.K. Holley, S.K. Dhar, Y. Xu, D.K. St Clair, Amino Acids (in press).
[39] R.G. Cutler, Basic Life Sci. 35 (1985) 15–73.
[40] J. Sun, D. Folk, T.J. Bradley, J. Tower, Genetics 161 (2002) 661–672.
[41] R. Doonan, J.J. McElwee, F. Matthijssens, G.A. Walker, K. Houthoofd, P. Back, A. Matscheski, J.R. Vanfleteren, D. Gems, Genes Dev. 22 (2008) 3236–3241.
[42] M. Murawski, J. Saczko, A. Marcinkowska, A. Chwilkowska, M. Grybos, T. Banas, Folia Histochem. Cytobiol. 45 (Suppl. 1) (2007) S123–S126.
[43] A. Florkowski, W. Gruszczynski, Z. Wawrzyniak, Mil. Med. 166 (2001) 375–377.
[44] A. Bergmann, J. Struck, M. Uhlein, N.G. Morgenthaler, US patent application 20090011431, 2009.
[45] T. Naganuma, T. Nakayama, N. Sato, Z. Fu, M. Soma, N. Aoi, R. Usami, Hypertens. Res. 31 (2008) 1533–1540.
[46] H. Fujimoto, H. Kobayashi, K. Ogasawara, M. Yamakado, M. Ohno, J. Cardiol. 55 (2010) 205–210.
[47] M.A. Bewick, M.S. Conlon, R.M. Lafrenie, Breast Cancer Res. Treat. 111 (2008) 93–101.
[48] P.G. Reeves, N.V. Ralston, J.P. Idso, H.C. Lukaski, J. Nutr. 134 (2004) 416–425.
[49] R. Thaler, H. Karlic, P. Rust, A.G. Haslberger, Br. J. Nutr. 101 (2009) 743–749.
[50] R.M. Lebovitz, H. Zhang, H. Vogel, J. Cartwright Jr., L. Dionne, N. Lu, S. Huang, M.M. Matzuk, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 9782–9787.
[51] A. Paul, A. Belton, S. Nag, I. Martin, M.S. Grotewiel, A. Duttaroy, Mech. Ageing Dev. 128 (2007) 706–716.
[52] L.A. Macmillan-Crow, D.L. Cruthirds, Free Radic. Res. 34 (2001) 325–336.
[53] S. Abba, H.R. Khouja, E. Martino, D.B. Archer, S. Perotto, Mol. Plant Microbe Interact. 22 (2009) 1412–1421.
[54] R. Sahoo, S. Kumar, P.S. Ahuja, J. Plant Physiol. 158 (2001) 1093–1097.
[55] B. Thomas, H.M. Davies, J. Kridl, J.K. O'Neal, C.J. Van Assche, US patent 5538878, 1996.
[56] T. Gill, Y. Sreenivasulu, S. Kumar, P.S. Ahuja, J. Plant Physiol, 167 (2010) 757–760.
[57] M.J. Morgan, M. Lehmann, M. Schwarzlander, C.J. Baxter, A. Sienkiewicz-Porzucek, T.C. Williams, N. Schauer, A.R. Fernie, M.D. Fricker, R.G. Ratcliffe, L.J. Sweetlove, I. Finkemeier, Plant Physiol. 147 (2008) 101–114.
[58] M.P. Lesser, W.R. Stochaj, Appl. Environ. Microbiol. 56 (1990) 1530–1535.
[59] D.J. Thomas, J.B. Thomas, S.D. Prier, N.E. Nasso, S.K. Herbert, Plant Physiol. 120 (1999) 275–282.
[60] J.A. Imlay, I. Fridovich, J. Bacteriol. 174 (1992) 953–961.
[61] G. St John, H.M. Steinman, J. Bacteriol. 178 (1996) 1578–1584.
[62] A. Battistoni, G. Donnarumma, R. Greco, P. Valenti, G. Rotilio, Biochem. Biophys. Res. Commun. 243 (1998) 804–807.
[63] X. Qi, A.S. Lewin, L. Sun, W.W. Hauswirth, J. Guy, Ann. Neurol. 56 (2004) 182–191.
[64] T.J. Bivalacqua, J.S. Armstrong, J. Biggerstaff, A.B. Abdel-Mageed, P.J. Kadowitz, W.J. Hellstrom, H.C. Champion, Am. J. Physiol. Heart Circ. Physiol. 284 (2003) H1408–1421.
[65] L. Agrawal, J.P. Louboutin, D.S. Strayer, Virology 363 (2007) 462–472.
[66] W.G. Sun, C.J. Weydert, Y. Zhang, L. Yu, J. Liu, D.R. Spitz, J.J. Cullen, L.W. Oberley, Cancers (Basel) 2 (2010) 68–87.
[67] J.S. Thompson, Y. Chu, J. Glass, A.A. Tapp, S.A. Brown, Free Radic. Res. 44 (2010) 529–540.
[68] R.F. Foronjy, O. Mirochnitchenko, O. Propokenko, V. Lemaitre, Y. Jia, M. Inouye, Y. Okada, J.M. D'Armiento, Am. J. Respir. Crit. Care Med. 173 (2006) 623–631.
[69] N.N. Ge, H.A. Brodie, S.P. Tinling, Otol. Neurotol. 29 (2008) 1061–1067.
[70] Y. Zhu, A.L. Kalen, L. Li, H.J. Lehmler, L.W. Robertson, P.C. Goswami, D.R. Spitz, N. Aykin-Burns, Free Radic. Biol. Med. 47 (2009) 1762–1771.
[71] K.R. Baumgardner, M.A. Sulfaro, J. Endodont. 27 (2001) 190–195.
[72] A. Bafana, S. Dutt, S. Kumar, P.S. Ahuja, Crit. Rev. Biotechnol. (in press).
[73] C. Tsao, P. Greene, B. Odlind, D.C. Brater, Clin. Pharmacol. Ther. 50 (1991) 713–720.
[74] D.V. Ratnam, D.D. Ankola, V. Bhardwaj, D.K. Sahana, M.N. Kumar, J. Control. Release 113 (2006) 189–207.
[75] F.M. Veronese, P. Caliceti, O. Schiavon, M. Sergi, Adv. Drug Deliv. Rev. 54 (2002) 587–606.
[76] R.A. Hallewell, I. Laria, A. Tabrizi, G. Carlin, E.D. Getzoff, J.A. Tainer, L.S. Cousens, G.T. Mullenbach, J. Biol. Chem. 264 (1989) 5260–5268.
[77] K. Mihara, K. Sawai, Y. Takakura, M. Hashida, Biol. Pharm. Bull. 17 (1994) 296–301.
[78] D. Hernandez-Saavedra, H. Zhou, J.M. McCord, Biomed. Pharmacother. 59 (2005) 204–208.
[79] M. Doroudchi, US patent application 20080181872, 2008.
[80] I. Vouldoukis, V. Sivan, M.C. Vozenin, C. Kamate, A. Calenda, D. Mazier, B. Dugas, Mol. Med. 6 (2000) 1042–1053.
[81] N. Sahgal, J.M. Davis, C. Robbins, S. Horowitz, E.G. Langenback, R.H. Perry, D. Colflesh, J. Tierney, S.R. Simon, Am. J. Physiol. 271 (1996) L230–L235.
[82] I. Ratcheva, Z. Stefanova, A. Vesselinova, S. Nikolova, A. Kujumdjieva, H. Neychev, Pharmazie 55 (2000) 533–537.
[83] M. Sanchez-Moreno, M.A. Garcia-Ruiz, A. Sanchez-Navas, M. Monteoliva, Comp. Biochem. Physiol. B 92 (1989) 737–740.
[84] P.M. Ekanayake, H.S. Kang, M. De Zyosa, Y. Jee, Y.H. Lee, J. Lee, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 145 (2006) 318–324.
[85] M.W. Lin, M.T. Lin, C.T. Lin, J. Agric. Food Chem. 50 (2002) 7264–7270.
[86] R. Khanna-Chopra, S. Sabarinath, Biochem. Biophys. Res. Commun. 320 (2004) 1187–1192.
[87] S. Kochhar, V.K. Kochhar, Planta 228 (2008) 307–318.
[88] H.J. Lee, H.W. Kwon, J.U. Koh, D.K. Lee, J.Y. Moon, K.H. Kong, J. Microbiol. Biotechnol. 20 (2010) 727–731.
[89] J. Yu, X. Yu, J. Liu, FEBS Lett. 562 (2004) 22–26.
[90] X. Wang, H. Yang, L. Ruan, X. Liu, F. Li, X. Xu, J. Ind. Microbiol. Biotechnol. 35 (2008) 133–139.
[91] C.F. Ken, C.C. Lee, K.J. Duan, C.T. Lin, Protein Exp. Purif. 40 (2005) 42–50.
[92] M.D. Holdom, B. Lechenne, R.J. Hay, A.J. Hamilton, M. Monod, J. Clin. Microbiol. 38 (2000) 558–562.
[93] N.N. Song, Y. Zheng, S.J. E, D.C. Li, J. Microbiol. 47 (2009) 123–130.
[94] H. Marberger, W. Huber, G. Bartsch, T. Schulte, P. Swoboda, Int. Urol. Nephrol. 6 (1974) 61–74.
[95] U. Muller, G. Moll, Z. Rheumatol. 42 (1983) 21–24.
[96] K.M. Goebel, U. Storck, Am. J. Med. 74 (1983) 124–128.
[97] H. McIlwain, J.C. Silverfield, D.E. Cheatum, J. Poiley, J. Taborn, T. Ignaczak, C.V. Multz, Am. J. Med. 87 (1989) 295–300.
[98] Y. Mizushima, K. Hoshi, A. Yanagawa, K. Takano, Drugs Exp. Clin. Res. 17 (1991) 127–131.
[99] Y. Niwa, K. Somiya, A.M. Michelson, K. Puget, Free Radic. Res. Commun. 1 (1985) 137–153.
[100] I.V. Churilova, E.V. Zinov'ev, B.A. Paramonov, Y.I. Drozdova, V.O. Sidel'nikov, V.Y. Chebotarev, Bull. Exp. Biol. Med. 134 (2002) 454–456.
[101] Y. Niwa, Dermatologica 179 (1989) 101–106.
[102] J. Emerit, S. Pelletier, J. Likforman, C. Pasquier, A. Thuillier, Free Radic. Res. Commun. 12-13 (Pt 2) (1991) 563–569.
[103] I. Marzi, V. Buhren, A. Schuttler, O. Trentz, J. Trauma 35 (1993) 110–120.
[104] W. Land, H. Schneeberger, S. Schleibner, W.D. Illner, D. Abendroth, G. Rutili, K.E. Arfors, K. Messmer, Transplantation 57 (1994) 211–217.
[105] T. Takemura, Nippon Kyobu Geka Gakkai Zasshi 41 (1993) 247–253.
[106] S. Delanian, F. Baillet, J. Huart, J.L. Lefaix, C. Maulard, M. Housset, Radiother. Oncol. 32 (1994) 12–20.
[107] Y. Lin, H.D. Pape, R. Friedrich, Int. J. Oral Maxillofac. Surg. 23 (1994) 428–429.
[108] J.M. Davis, R.B. Parad, T. Michele, E. Allred, A. Price, W. Rosenfeld, Pediatrics 111 (2003) 469–476.
[109] S. Shimmura, R. Igarashi, H. Yaguchi, Y. Ohashi, J. Shimazaki, K. Tsubota, Am. J. Ophthalmol. 135 (2003) 613–619.
[110] Y. Suzuki, T. Matsumoto, S. Okamoto, T. Hibi, Colorectal Dis. 10 (2008) 931–934.
[111] C.R. Riedl, P. Sternig, G. Galle, F. Langmann, B. Vcelar, K. Vorauer, A. Wagner, H. Katinger, H. Pfluger, Eur. Urol. 48 (2005) 656–661.
[112] G. Sanclemente, J.J. Garcia, J.J. Zuleta, C. Diehl, C. Correa, R. Falabella, J. Eur. Acad. Dermatol. Venereol. 22 (2008) 1359–1364.
[113] L.Z. Stern, S.P. Ringel, F.A. Ziter, K.B. Menander-Huber, V. Ionasescu, R.J. Pellegrino, R.D. Snyder, Arch. Neurol. 39 (1982) 342–346.
[114] O.S. Nielsen, J. Overgaard, M. Overgaard, S. Steenholdt, A. Jakobsen, A. Sell, Acta Oncol. 26 (1987) 101–104.
[115] J.T. Flaherty, B. Pitt, J.W. Gruber, R.R. Heuser, D.A. Rothbaum, L.R. Burwell, B.S. George, D.J. Kereiakes, D. Deitchman, N. Gustafson, et al., Circulation 89 (1994) 1982–1991.
[116] R. Pollak, J.H. Andrisevic, M.S. Maddux, S.A. Gruber, M.S. Paller, Transplantation 55 (1993) 57–60.
[117] C.E. Garcia, C.M. Kilcoyne, C. Cardillo, R.O. Cannon 3rd, A.A. Quyyumi, J.A. Panza, Hypertension 26 (1995) 863–868.
[118] B. Young, J.W. Runge, K.S. Waxman, T. Harrington, J. Wilberger, J.P. Muizelaar, A. Boddy, J.W. Kupiec, JAMA 276 (1996) 538–543.
[119] M.E. Cudkowicz, L. Warren, J.W. Francis, K.J. Lloyd, R.M. Friedlander, L.F. Borges, N. Kassem, T.L. Munsat, R.H. Brown Jr., Neurology 49 (1997) 213–222.
[120] J. Butterworth, C. Legault, D.A. Stump, L. Coker, J.W. Hammon Jr., B.T. Troost, R.L. Royster, D.S. Prough, J. Cardiothorac. Vasc. Anesth. 13 (1999) 690–694.
[121] E.P. Yuksel, F. Aydin, N. Senturk, T. Canturk, A.Y. Turanli, Eur. J. Dermatol. 19 (2009) 341–344.
[122] C.M. Muth, Y. Glenz, M. Klaus, P. Radermacher, G. Speit, X. Leverve, Free Radic. Res. 38 (2004) 927–932.
[123] M. Cloarec, P. Caillard, J.C. Provost, J.M. Dever, Y. Elbeze, N. Zamaria, Eur. Ann. Allergy Clin. Immunol. 39 (2007) 45–50.
[124] M.A. Milesi, D. Lacan, H. Brosse, D. Desor, C. Notin, Nutr. J. 8 (2009) 40.