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Review

Superoxide dismutases and their impact upon human health

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Abstract

Superoxide dismutases (SOD), a group of metal-containing enzymes, have a vital anti-oxidant role in human health, conferred by their scavenging of one of the reactive oxygen species, superoxide anion. Three types of SODs are known in humans, with the most abundant being cytosolic SOD1, identified by its Cu, Zn-containing prosthetic group. The presence of these metals and the coordination to certain amino acids are essential for function. SODs are among the first line of defense in the detoxification of products resulting from oxidative stress. Here, we describe the importance of SOD function, and the need for coordination with other ROS-scavenging enzymes in this pathway of detoxification. The impact of metal-deficient diets (copper or zinc) or incorrect metal ion incorporation (copper chaperone for SOD) onto nascent SOD, are also examined. Finally, human pathologies associated with either SOD dysfunction or decreased activity are discussed with current progress on the development of novel therapies.

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Abbreviations: SOD, superoxide dismutase; ROS, reactive oxygen species; CCS, copper chaperone for SOD; MTM1, manganese trafficking factor 1; PUFA, polyunsaturated fatty acids; ALS, amyotrophic lateral sclerosis

Keywords: Reactive oxygen species; Reactive nitrogen species; Copper-deficiency; Zinc-deficiency

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1. Introduction

Superoxide dismutases (SODs) are metalloenzymes found widely distributed in prokaryotic and eukaryotic cells (Fridovich, 1995). They constitute an enzyme family that catalyzes the conversion of superoxide anion to hydrogen peroxide (H_2O_2). The two-step chemical reaction of superoxide anion with the prosthetic group of SOD begins with the oxidized form of the enzyme (Fe^{3+} , Cu^{2+} and Mn^{3+} respectively) binding superoxide anion, acquiring a proton and releasing molecular oxygen. The reduced form of the enzyme (Fe^{2+} , Cu^+ and Mn^{2+}) then binds a second superoxide anion and proton, to liberate H_2O_2 and return to its oxidized state. Despite the metal ion centre becoming more negatively charged, the binding of the second superoxide anion is possible in an environment of neutral pH, by the total charge of the active site remaining the same.

Depending on species, there may be up to three different metal-containing SOD enzymes present which, taken together, make up the major superoxide scavenging system in the mitochondrion, nucleus, cytoplasm and extracellular spaces. These SODs are the products of different genes and are historically designated, in higher eukaryotes, by their primary location as follows: SOD1 (cytoplasmic), SOD2 (mitochondrial) and SOD3 (extracellular). The SOD3 may bind to cell surfaces by its interaction with polyanions, such as heparan sulphate. SOD3 has been characterized in three different forms, namely SOD3A (no heparin affinity), SOD3B (low heparin affinity) and SOD3C (high heparin affinity) (Marklund, 1984). Although present in comparatively lower amounts, it is from the C-terminal region of high-heparin affinity SOD3 that has been exploited in the use of SOD for therapeutic means (see section on future therapeutic strategies).

In humans, the SOD family members are either dimeric (SOD1; 32 kD, McCord and Fridovich, 1969) or tetrameric (SOD2; 89 kD, McCord, 1976, SOD3; 135 kD, Marklund, 1982). They are also defined between kingdoms by virtue of their bound metal ion, namely the Cu, Zn-SOD and Mn-SOD class or the Fe-SOD class, the latter of which exists in prokaryotic cells and some green plants. The discovery of

a Ni-SOD in *Streptomyces* and cyanobacteria established a completely new SOD group with a unique Ni-hook motif (Youn et al., 1996). Although all SODs catalyze the same reaction (i.e., the dismutation of superoxide anion), they do not share a significant primary structure homology (Fig. 1, compare Cu, Zn-, with Ni- or Fe/Mn-containing SOD). Only Fe-SOD and Mn-SOD share similarities in protein folding (Fig. 1). For a more detailed discussion on this topic refer to Miller (2004).

The SODs are one part of a suite of enzymes that catalyzes reactive oxygen species (ROS), produced as minor by-products of metabolism, to less reactive species (Fig. 2). Oxidative stress arises as a result of an enhanced production of ROS or a deficient anti-oxidant system (enzymatic and non-enzymatic). This leads to the chemical modification of key cellular components. Some known and well-studied consequences of oxidative stress are alteration of membrane integrity, DNA instability and decline in enzymatic activities. In terms of the whole organism, increased oxidative stress has been associated with many acute and chronic pathologies. An example of an acute pathology is ischemia-reperfusion injury (Crack and Taylor, 2005) whereas for chronic pathologies, it may be vascular damage in patients with diabetes mellitus (Oberley, 1988). Oxidative stress is also associated with the aging process (for a review see Landis and Tower, 2005).

Part of the cell's stress response is to increase the transcription of the *sod* genes, which leads to increased activity in those regions in most need of protection by

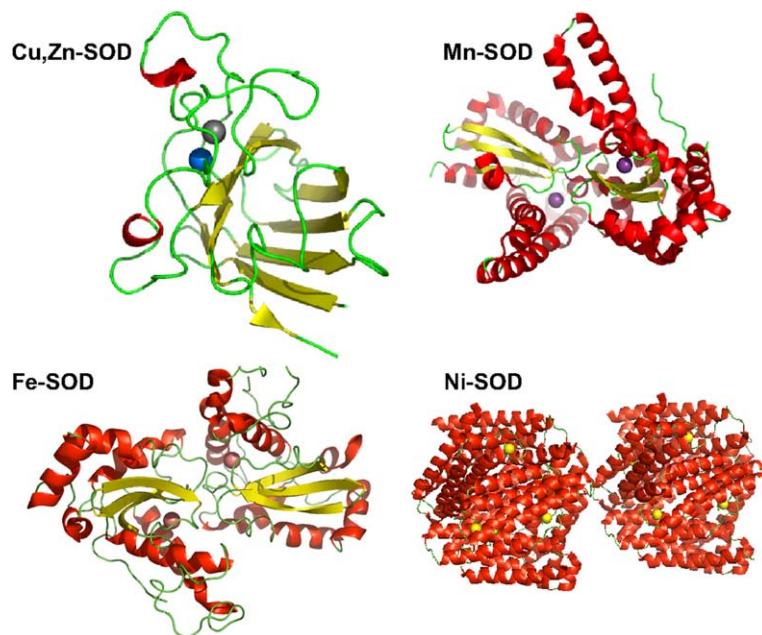


Fig. 1. 3D-structures of SOD with various metal ligands required for its activity and/or preservation of structure. The models were obtained by using the following files from the pdb: Cu, Zn-SOD (1B4T.pdb), Mn-SOD (N0J.pdb); Fe-SOD (3SDP.pdb), and Ni-SOD (1T6U.pdb), and were represented with Pymol version 0.98.

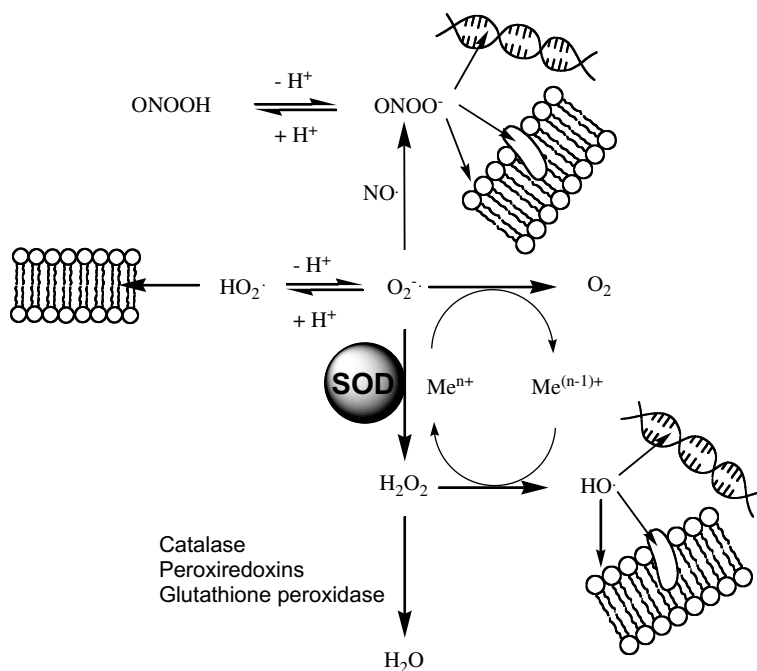


Fig. 2. Role of superoxide dismutase (SOD) in the reactive oxygen species scavenging pathway.

anti-oxidants. This has been shown gene expression profiles using a number of different tissues, under different stress conditions (McMillian et al., 2004; Nilakantan et al., 2005). Superoxide anion can also react with nitric oxide to form the powerful oxidant peroxynitrite (OONO^- ; Fig. 2). The radius of reaction for this species is limited by its high reactivity and charge. However, its conjugated acid form (OONOH) may cross membranes reaching other cellular compartments (Moller et al., 2005; Denicola et al., 2002, 1998).

It is expected that superoxide anion, disproportionated by SOD, immediately becomes a substrate for the enzyme catalase and other hydrogen peroxide-catalyzing enzymes such as glutathione peroxidases and peroxiredoxins (Fig. 2). However, the conversion of superoxide anion to hydrogen peroxide by SOD may be viewed as a Janus effect, with anti-oxidant and prooxidant consequences. On the one hand, the dismutation of superoxide anion, a species which is negatively-charged and therefore membrane-impermeable, to H_2O_2 and oxygen, both diffusible species, facilitates both the distribution of ROS, i.e. diluting their effects via diffusion between cellular compartments, and the removal of H_2O_2 by H_2O_2 -consuming enzymes (anti-oxidant). On the other hand, if the actions of SOD and H_2O_2 -consuming enzymes are not in concert, an increased production of H_2O_2 is expected from SOD activity. This may facilitate the production of hydroxyl radical and the consequential damage, if an appropriate metal-bound species for this reaction is found.

2. SOD synthesis, targeting and assembly

All of the mammalian SODs are nuclear-encoded, being initially formed as inactive apo-enzymes. For fully functional mitochondrial MnSOD (SOD2), yeast cells require the nascent polypeptide to be targeted to the mitochondrial membrane, where it is folded and correctly receives its manganese prosthetic group (Luk et al., 2005). Pre-folded peptides, or those that have accumulated in the cytosol, cannot be efficiently transported, folded and loaded with manganese. The manganese trafficking factor (MTM1) is the key player in the mitochondrial matrix that allows the conversion of the polypeptide into an active holoenzyme. When MTM1 is inactivated in yeast, SOD2 activity can only be restored if cells are supplemented with high doses of manganese (Luk et al., 2003). A small fraction of the abundant cytosolic SOD1 has been shown to reside in the mitochondrial inter-membrane space (IMS) (Sturtz et al., 2001). As with SOD2, this small amount of SOD1 can only enter the mitochondria as an apo-enzyme. A copper chaperone for SOD1 (CCS) converts the apo-SOD1 into its fully functional holoenzyme (Wong et al., 2000). Once inside the IMS, the CCS (which is present in high amounts) is responsible for the copper loading of the enzyme (Field et al., 2003) which occurs via a physical interaction between the SOD1 and CCS, involving sequences in the latter's protein domain III. Both proteins contain a homologous central protein domain II that is also required for successful interaction and copper loading of SOD1 (Schmidt et al., 2000). Interestingly, CCS itself has almost every metal-binding ligand that is present in SOD1, however it does not exhibit the ROS-scavenging activity. Schmidt et al. (1999a,b) mutated the single ligand difference in CCS to be fully homologous to the equivalent SOD1 ligand, and revealed a novel SOD activity by this mutated yeast copper chaperone. Experiments such as these, demonstrate the importance of the active site preservation along with its ability to maintain the same overall charge but with its re-distribution allowing catalysis.

In the mammalian system to further study the role of CCS, Wong et al. (2000) generated CCS-inactivated (CCS $-/-$) mice. Various tissues (brain, spinal cord, muscle, liver, lung, heart and kidney) from these animals revealed normal levels of SOD1 protein, concomitant with SOD1 activity that was significantly lower than those of their littermates. This research has important implications in the familial amyotrophic lateral sclerosis (FALS) where mutations within the *sod1* gene in FALS individuals have been attributed to a "gain of function" toxicity, rather than a loss of function trait (Gurney et al., 1994). The results of Wong et al. (2000) and others (that show CCS and SOD1 interactions occurring in mammals are not unlike those in yeast) open up the research field to further investigate the potential aberrant copper chemistry in ALS (as described below).

3. SOD dysfunction and metal ion deficiencies

Copper is essential in the human diet because it is involved in the proper utilization of iron and especially for the synthesis of important biomolecules such as Cu,

Zn-SOD and cytochrome oxidase, and Fe- and Cu-containing protein. Metal deficiencies of copper, zinc or manganese, as a direct result of diets devoid of these elements, are very rare in humans and almost absent in western cultures. More common are conditions such as protein malnutrition that lead to deficiencies of certain micronutrients. Aberrant uptake by virtue of genetic mutations is also rare. However two hereditary diseases that affect the levels of copper in the body are Wilson's and Menke's diseases. The former represents a disease where copper is not excreted from the body, resulting in gradual accumulation leading to the inherent symptoms of copper toxicity, such as liver disease (Brewer and Askari, 2005). Menke's disease is a copper-deficient syndrome involving faulty copper transport. In this case the symptoms usually include hypopigmentation, pili torti (kinky hair), fragile bones, vascular abnormalities (such as aneurysms) and severe neuropathology. Patients with Menke's disease rarely live beyond 3–4 years of age (Mercer, 2001; Barnes et al., 2005). In order to study metal ion deficiencies and their effects on SOD function, rodent models have been used with strict dietary control. Concern is also taken to include certain amino acids in metal deficient diets.

4. Copper-deficient diets

In a study by Reeves et al. (2004) male rats, fed a diet with and without zinc, copper or manganese (plus either L-Cys or Met supplements), showed symptoms with their copper-deficient diet. There was decreased activity in red blood cell SOD1, hepatic cytochrome oxidase and serum SOD3 activity, each being only 14%, 25% and 20% respectively of that in the control animals (Reeves et al., 2004). Earlier studies, in which rats were kept for 4-weeks on a copper-deficient diet, revealed selective and organ-specific regulation of Mn-SOD and Cu, Zn-SOD. In the heart, Cu, Zn-SOD activity was diminished despite a high expression of both transcript and apoprotein. Cardiac Mn-SOD was elevated in activity and protein levels with no change at the transcript level, suggesting a slower turnover of the holoprotein. In the liver, the decrease in Cu, Zn-SOD activity was accompanied by a decrease in transcripts and protein levels, indicating regulation was primarily at the transcription level. Mn-SOD transcript levels in liver were much greater than the controls, supportive of the induction of *sod2* gene expression. Finally, in the brains of the same animals there was no effect of restricting dietary copper (Lai et al., 1994). This study showed that dietary deficiencies of micronutrients such as copper, may result in changes in SOD transcription, translation, or protein synthesis levels, depending on the organ.

5. Zinc-deficient diets

Although zinc is not involved directly in the enzymatic activity of Cu, Zn-SOD (i.e., it is not part of the catalysis), it is important because it is required to maintain the protein structure (Oberley, 1976). Oteiza et al. (1996) studied the effect on testicular Cu, Zn-SOD activity by changing the diet of young rats from a zinc-adequate to

zinc-deficient one. They found, surprisingly, that the activity became 34% higher in the zinc-deficient animals compared to the zinc-adequate controls. This was believed to be a compensatory change, owing to the increased oxidative stress initially experienced after a 2-week interval of zinc-deprived conditions. The enforced dietary restriction had no effect on Mn-SOD activity during the same period. Taking a more long-term diet approach, Ozata et al. (2002) studied 76 obese human males and their age-matched controls. They found that, in the obese individuals, lower zinc (but not copper or iron) occurred along with less activity of Cu, Zn-SOD and glutathione peroxidase. In addition, the level of red blood cell thiobarbituric acid reactive substances, an indicator of oxidative stress, was higher in the obese males, indicating the pathological changes associated with obesity could be due to deficient ROS scavenging enzymes.

6. Metal ion toxicity

More often, metal ion toxicity is of greater concern with the progression of pathological conditions. Excessive zinc and oxidative stress are two important players in the formation of amyloid plaques in the neuronal tissue of Alzheimer patients (for a review see Cuajungco and Faget, 2003). In the case of Parkinson's disease, the copper released from Cu, Zn-SOD (after oxidative damage to this enzyme) is believed to accelerate the aggregation of α -synuclein, the main protein constituent in Lewy bodies and also present in the plaques of Alzheimer's Disease (Kim et al., 2002). Other studies to investigate mechanisms to protect the neuron from aberrant zinc levels have focused on omega-3 polyunsaturated fatty acids (PUFA). Omega-3 PUFA, provided in the diet of rats, can modify zinc homeostasis in the brain tissue and decrease the incidence of ensuing oxidative damage (Jayasooriya et al., 2005). In the ALS mutant mouse model, it is known that high dietary zinc amplifies toxicity. Ermilova et al. (2005) used this system to show that the higher zinc levels were inhibiting copper absorption, and producing lethal anemia. They studied animals with a moderate and high zinc regime (12 and 18 mg/kg/day, respectively) with a third group receiving the high zinc plus 0.3 mg/kg/day copper supplements. The moderate zinc regime increased the ALS mouse life span by 11 days, while the copper-supplement in the high zinc regime prevented the early death seen with high zinc alone.

7. SOD-related human pathologies

A decrease in SOD activity may be caused by a number of factors. Only some cases within the genetic disorders, such as ALS (see below for details), can be diagnosed as SOD dysfunction-related, human pathologies. As already described, metal ion deficiencies contribute to the dysfunction and lowered activity of a suite of enzymes, and the effects may be age- and/or organ-specific. Pathological conditions, such as obesity, have been shown to contribute to SOD dysfunction. In the rat model system for diabetes, animals displayed aberrant copper homeostasis that led to lower

Cu, Zn-SOD activity. The lower copper retention and SOD activity was further exacerbated by imposing a copper-deficient diet (Uriu-Adams et al., 2005 abstract only). This indicates the complexity of SOD-related human pathologies arising from various causes. At the basis of other proliferative diseases, such as hepatocellular carcinoma, is the deficiency of the SOD enzyme during a particular stage of development. This has been shown in human cases as well as being recapitulated in mouse model systems (Liaw et al., 1997 abstract only). Mice deficient in Cu, Zn-SOD (*sod1* $-/-$) did not appear abnormal in their development until adulthood. However, their life span was reduced compared with wild-type mice, and they exhibited increased neoplastic changes in the liver (Elchuri et al., 2005). Cancer cells generally have diminished activity of both Cu, Zn-SOD and Mn-SOD (Oberley, 2004; St Clair et al., 2005).

Amyotrophic lateral sclerosis (ALS) or “Lou Gehrig’s Disease” is a selective, neuro-degenerative disease of the motor neurons in the spinal cord and brain. Signs and symptoms include generalized weakness and muscle atrophy with progressive paralysis. Approximately 10% of cases are familial, with the other cases being sporadic. The selective death of these neurons is caused by a dominant mutation in the gene *sod1*. Over 90 distinct mutations have been identified, however only 2–3% of the familial cases have matched the identities of these mutations (Beckman et al., 2002). Of the small percentage of familial cases that carry known mutations the destabilization of SOD1 can diminish zinc affinity by upto 50-fold, compared to wild-type (Crow et al., 1997). To further complicate this syndrome, numerous studies have indicated that the mutant form of SOD1 can exhibit novel toxic properties (Gurney et al., 1994; Crow et al., 1997). Over-expression of mutant *sod1* in the mouse model ALS systems gave rise to progressive paralysis and death by 120 days of age. This toxic gain of function may be due, in part, by the mutation not only undermining SOD’s protective role but also by the mutant SOD1’s ability to trigger deleterious reactions (Gurney et al., 1994; Furukawa and O’Halloran, 2005).

Estevez et al. (1999) investigated the effects on SOD1 when copper coordination in the active site was altered by zinc depletion. Using liposomal delivery they introduced Cu, Zn-SOD1 (in both wild-type and zinc-replete ALS mutant form) and zinc-depleted SOD1 into cultured neurons and evaluated toxic effects. They surmised that it was zinc-depletion that caused SOD1 to trigger apoptosis. Neither the wild-type nor ALS SOD1 forms were toxic to the cells when in a zinc-replete state. They hypothesized that the SOD1 active site in the ALS zinc-depleted mutant favored the backward reaction, the conversion of oxygen to superoxide anion. This in turn would promote peroxynitrite formation, as the superoxide would combine with nitric oxide, explaining the toxic gain-of-function in ALS via nitrative damage (Estevez et al., 1999).

Williamson et al. (2000) argue that none of the known ALS mutations involve zinc-binding residues. Furthermore, if the hypotheses of Estevez et al. (1999) were correct, transgenic mice with both the ALS SOD1 mutations as well as a disrupted nNOS gene (leading to a 14-fold decreased NOS’ activity) should show less symptoms or a delayed onset of the disease. However, according to Williamson et al. (2000), this is not the case, suggesting that more research needs to be performed in this area.

Recently, a role for the copper chaperone for SOD1 (CCS) has emerged as a likely defect in ALS. Initially, there were some indications that CCS might be involved in the formation of the cytosolic protein aggregates and inclusions common to both familial and sporadic ALS. This would place CCS in the group of potential targets for ALS therapy. However, in [Watanabe et al. \(2001\)](#) found, in the tissue of the three different mouse ALS models, that while all of the aggregates were CCS-positive, not all of the inclusions were shown to either contain SOD1 or to be CCS-positive. Ironically these and other studies focused on CCS may have given us more information on SOD1 function, than on potential ALS treatments. The transgenic mouse CCS (–/–) mutant, who failed to produce the functional copper chaperone for SOD1, exhibited a 15% SOD1 activity (a CCS-independent SOD1 activity) in comparison to wild-type controls ([Wong et al., 2000](#)). In this regard, [Beckman et al. \(2002\)](#) have suggested that copper could be provided by other means than CCS in these knockout mice.

The research described above indicates the complexity of the different SOD1 mutations and their effects within the syndrome of ALS. While a cure for the disease has not yet been achieved, the results from these efforts will assist in the development of potential therapies.

8. Current and future therapeutic strategies

Treatments currently being developed, to target the SOD role in scavenging ROS, may take one of several approaches. 1. To enhance the cell's SOD abilities, often using natural extracts ([Ng et al., 2005](#)). 2. SOD mimetics ([Izumi et al., 2002](#); [Pong et al., 2002](#); [Zhang et al., 2004](#)), and 3. Supplementary enzyme therapy. In the first case, the strategy is aimed at increasing native SOD activity and/or gene expression with or without additional anti-oxidant therapy. This may be with drugs that are already in common use, such as the anti-hypertensive, ACE inhibitors, which [Umemoto et al. \(2004\)](#) have shown can upregulate Cu, Zn-SOD activity in heart tissue. In the case of SOD mimetic drugs (still at the stage of animal trials) porphyrin-based or salen-manganese compounds, that can mimic either SOD or catalase activity (or both), are administered for the long term at protective doses.

Other approaches to therapy have shown greater promise, in particular the recent construction of a “super-SOD” ([Gao et al., 2003](#)). This chimeric protein contains SOD2 primary structure plus the 26 amino acid C-terminus of SOD3. This tail allows the SOD2/3 adherence to endothelial surfaces. With the chimera being less positively charged than SOD3C, this means it does not bind as strongly to cell surfaces, allowing its successful i.v. administration (for a review see [Hernandez-Saavedra et al., 2005](#)). When administered to rats, SOD2/3 was effective as an anti-inflammatory therapy during conditions of pulmonary injury and foot edema. The same chimera was also effective at decreasing the oxidative damage ensuing reperfusion in cats. In this recent study, [Bonder et al. \(2004\)](#), showed that SOD2/3 decreased the amount of neutrophil–endothelial cell interaction that under controlled conditions typically led to microvascular dysfunction.

9. Conclusions

Understanding the biological advantage conferred by an enzyme that converts one reactive oxygen species into another one (superoxide anion to hydrogen peroxide) was perplexing. It was clear that SOD played a critical role considering (i) its fast reaction with superoxide anion, (ii) its presence in every organ and almost all intracellular compartments and, (iii) the high concentration of SOD compared to its substrate i.e. SOD concentrations are 10^6 -times higher than that of superoxide anion (Giulivi et al., 1999). The answer was found in 1987 with the discovery of nitric oxide as an important cellular biomolecule (Palmer et al., 1987) and later with the work of Kissner et al. (1998) and others, where the rate of reaction between superoxide anion and nitric oxide was found to be the fastest in biological systems ($2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$). This explained the need for a high concentration of the enzyme that effectively would have to compete for superoxide anion when nitric oxide was present. This competition prevents the formation of peroxynitrite and the consequential damage that this species would have generated. Thus, there is a delicate balance between the concentrations of superoxide anion, nitric oxide, SOD and hydrogen peroxide-consuming enzymes.

Current research continues to investigate new strategies to enhance the ROS-scavenging systems of the cell, in an effort to attenuate the damage resulting from oxidative stress. SOD is one enzyme in the cell's defense against ROS. This defense system becomes increasingly important as more pathological and disease states are examined and it is discovered that oxidative damage is a cause, if not a critical player.

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