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## Pulse Radiolysis Study of Catalytic Properties of Human Manganese Superoxide Dismutase: A Review

Alhassan A. J. <sup>1\*</sup>, Muhammad, I. U. <sup>1</sup>, Sule, M.S <sup>1</sup>, Adamu, S.M. <sup>2</sup> and Umar, Y.<sup>3</sup>

Department of Biochemistry, Bayero University Kano, Nigeria <sup>1</sup>, Pathology Department, National Orthopaedic Hospital, Dala, Kano <sup>2</sup>, Department of Science Laboratory Technology, Federal College of Agricultural Produce Technology, Hoto, Kano <sup>3</sup>.

\* Correspondence Author: ajalhassan@yahoo.com

### Abstract

The depletion of superoxide catalyzed by human manganese superoxide dismutase (MnSOD) was observed spectrophotometrically by measuring the absorbance of superoxide at 250-280 nm following pulse radiolysis and it showed a biphasic pattern. Tryptophan 161 is a highly conserved residue that forms a hydrophobic side of the active site cavity of manganese superoxide dismutase (MnSOD), with its indole ring adjacent to and about 5 Å from the manganese. Trp 161 promotes the dissociation of product peroxide, perhaps in part through its effect on the orientation of Tyr 34.

**Key Words:** Manganese, Pulse Radiolysis, Superoxide dismutase, Tryptophan

### Introduction

A problem shared by all aerobic organisms is that between 1% and 3% of the oxygen consumed by respiration is partially reduced to reactive oxygen species (ROS) including superoxide, hydrogen peroxides, and hydroxyl radicals (Ines et al., 2005). Other in vivo sources of ROS include peroxisomal  $\beta$ -oxidation of fatty acids, microsomal cytochrome P450 metabolism of xenobiotic compounds (figure 1), and stimulation of phagocytosis by pathogens or lipopolysaccharides, arginine metabolism, and tissue specific enzymes. (Ray et al., 2012).

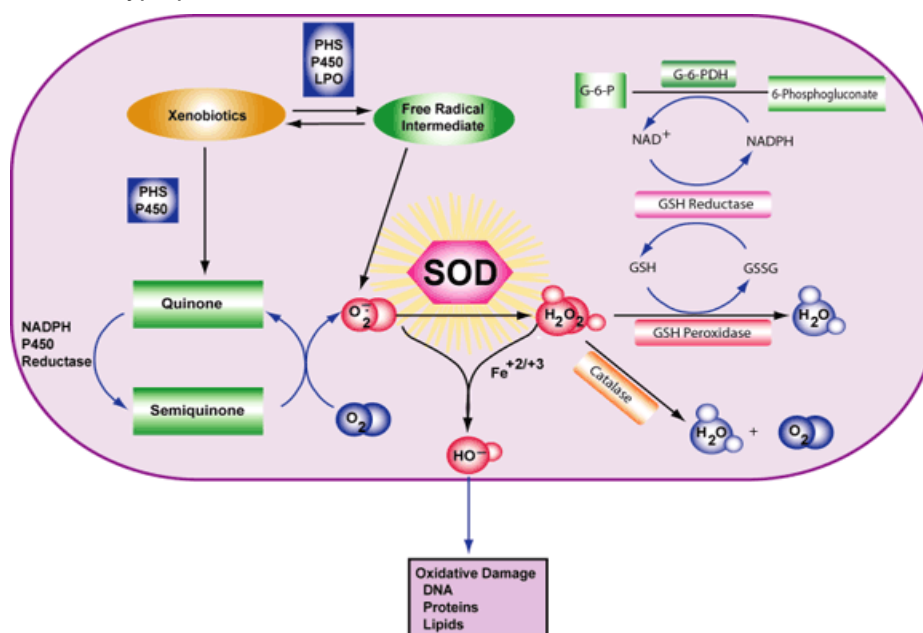


Fig 1: Systemic generation of superoxide

The main cells damage results from ROS-induced alteration of macromolecules such as polyunsaturated fatty acids in membrane lipids, essential proteins, and DNA. Additionally, oxidative stress and ROS have been implicated in disease states, such as Alzheimer's disease, Parkinson's disease, cancer, and aging (De La Torre et al., 1999).

Mammalian cells possess both non-enzymatic and enzymatic antioxidant defenses to cope with oxygen-free radicals. The former include a wide variety of such compounds as alpha-tocopherol (vitamin E), beta-carotene, and ascorbic acid (vitamin C), whereas the latter includes the scavenging enzymes; superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). These enzymes form an important defense system to ensure that cytotoxic oxygen species are degraded to less harmful compounds so that extensive cell damage does not occur (De La Torre et al., 1999).

**Superoxide dismutase** (SOD, EC 1.15.1.1), are proteins cofactored with either; copper and zinc, or manganese, iron, or nickel that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. As such, it is an important antioxidant defense in nearly all cells exposed to oxygen (Fridovich, 2010). The SOD-catalysed dismutation of superoxide may be written with the following half-reactions:

- $M^{(n+1)+} - SOD + O_2^- \rightarrow M^{n+} - SOD + O_2$
- $M^{n+} - SOD + O_2^- + 2H^+ \rightarrow M^{(n+1)+} - SOD + H_2O_2$

Where M = Cu (n=1), Mn (n=2), Fe (n=2), Ni (n=2).

In this reaction the oxidation state of the metal cation oscillates between n and n+1.

#### Types of SOD:

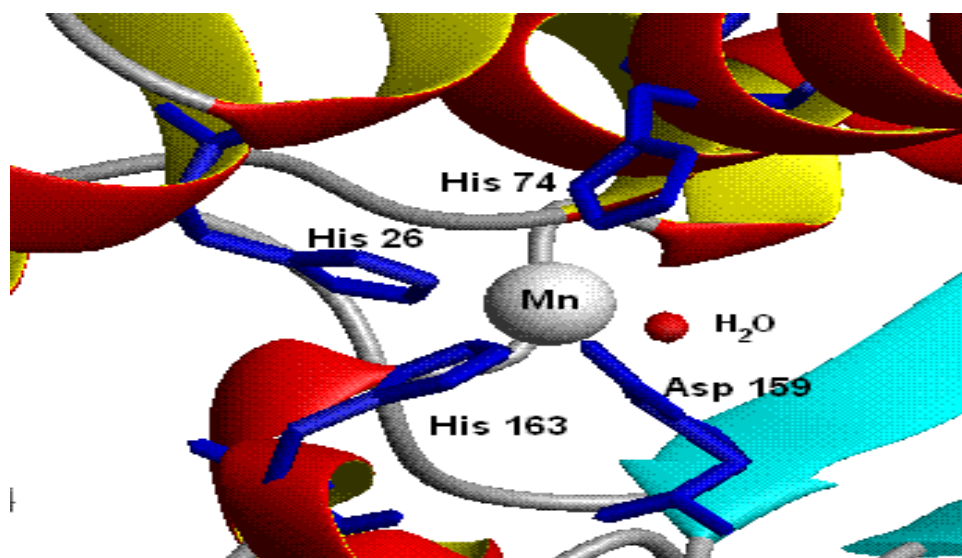
- Copper and zinc superoxide dismutase (Cu-Zn-SOD): It is found in the cytosols of virtually all eukaryotic cells. The Cu-Zn enzyme is a homodimer of molecular weight 32,500. The two subunits are joined primarily by hydrophobic and electrostatic interactions.
- Manganese superoxide dismutase (Mn-SOD): Chicken liver (and nearly all other) mitochondria and many bacteria (such as *E. coli*) contain a

form with manganese superoxide dismutase (Mn-SOD). The ligands of the manganese ions are 3 histidine side chains, an aspartate side chain and a water molecule or hydroxy ligand depending on the Mn oxidation state (respectively II and III).

- Iron superoxide dismutase (Fe-SOD): *E. coli* and many other bacteria also contain a form of the enzyme with iron (Fe-SOD); some bacteria contain Fe-SOD, others Mn-SOD, and some contain both. The active sites of Mn and Fe superoxide dismutase contain the same type of amino acids side chains.

All the three forms are present in humans and are designated as SOD1 located in the cytoplasm, SOD2 in the mitochondria and SOD3 is extracellular (Sentman, 2006). Other recent reports involving SOD knockouts have revealed that Mn-SOD is essential for life whereas Cu/Zn-SOD is not. Cu/Zn-SOD knock-out mice appear normal and exhibit differences only after traumatic injury, whereas Mn-SOD knockouts do not survive past 3 weeks of age. Among various human tissues Mn-SOD contents were roughly one-half as large as the Cu/Zn-SOD contents (Li et al., 2011).

The crystal structure of human MnSOD determined at 2.2 Å resolution shows that the active sites grouped in pairs across the dimer interfaces. The positions of the four amino acid ligands of the manganese (His26, His74, His163, and Asp159) form strained trigonal bipyramidal geometry on the manganese (Borgstahl et al., 1992). This is similar to MnSOD from *Thermus thermophilus* at 1.8 Å, and from *Bacillus stearothermophilus* at 2.4 Å. These structures are also similar to that of the FeSOD (Sentman, 2006). The superoxide anion binding site is located to the cleft close to the dimer interface. There are two regions that may control the traffic of substrate and product: one located near the Mn binding site, and another in the dimer interface. The evolutionary conservation of reciprocal interactions of both monomers in neighboring active sites suggests possible subunit cooperation during catalysis.



**Fig2: Active site of MnSOD, showing ligand residues to Mn.** (Borgstahl et al., 1996).

Initial studies using pulse radiolysis of the MnSOD determined that its catalysis was complicated by the presence of an inactive form of the enzyme that can interconvert to an active form (Robert et al., 1974). Steady-state constant obtained by Bull and colleagues (1991) from stopped-flow experiments and for the rapid emergence of the inactive form of the enzyme was quantitated. They were able to observe the inactive form spectrophotometrically and suggested that it results from oxidative addition of  $O_2^-$  to the Mn(II) form of the enzyme, resulting in a side-on complex of Mn(III) and the peroxide,  $Mn(III)O_2^-$ . Observation of catalysis by pulse radiolysis for  $[O_2^-]/[E^o]$  showed an initial burst of activity followed by the rapid emergence of an inhibited form of greatly reduced overall catalytic rate. The emergence of inhibition with human MnSOD is sufficiently rapid that the initial burst of catalysis by uninhibited enzyme is over in 1 or 2 ms and hence not able to be observed by stopped flow instrumentation. Human MnSOD rate of catalysis in

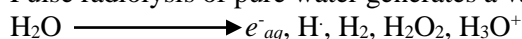
the zero order region is unaffected by changing the concentrations of several buffers and did not vary over the pH range 8.5 – 10.5. Proton transfer steps are rate contributing in the interconversion of active and inactive enzymes (IAEA, 2005).

#### **Pulse Radiolysis demonstration of Superoxide Dismutase catalytic action**

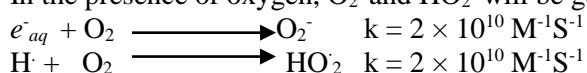
The enzymatic reaction was a first order with respect to  $O_2^-$  and the rate constant, which was  $2.3 \times 10^9 M^{-1}S^{-1}$  at pH 7.0 and 20 – 25°C, was not much affected by pH, in the range of 4.8 – 9. Because of instability of its substrate ( $O_2^-$ ), study of superoxide dismutase have depended largely upon the indirect assessments of its activity; such as its ability to inhibit  $O_2^-$  dependent reactions. However, it appeared possible to show directly and measure the catalytic action of superoxide dismutase by using pulse radiolysis to generate  $HO_2^-$  and  $O_2^-$  in aqueous solution (Sule, 2010).

#### **Radiolysis of Water**

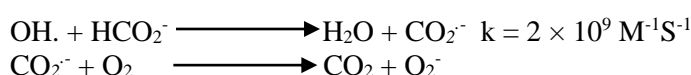
Pulse radiolysis of pure water generates a variety of products as:



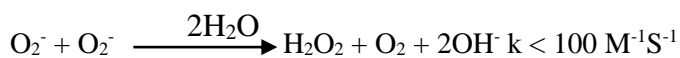
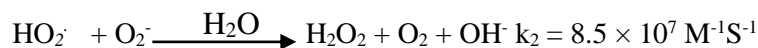
In the presence of oxygen,  $O_2^-$  and  $HO_2^-$  will be generated by the reactions;



Formate doubles the radiolytic yield of  $O_2^-$  by converting  $OH\cdot$  to  $O_2^-$  according to the following reactions;

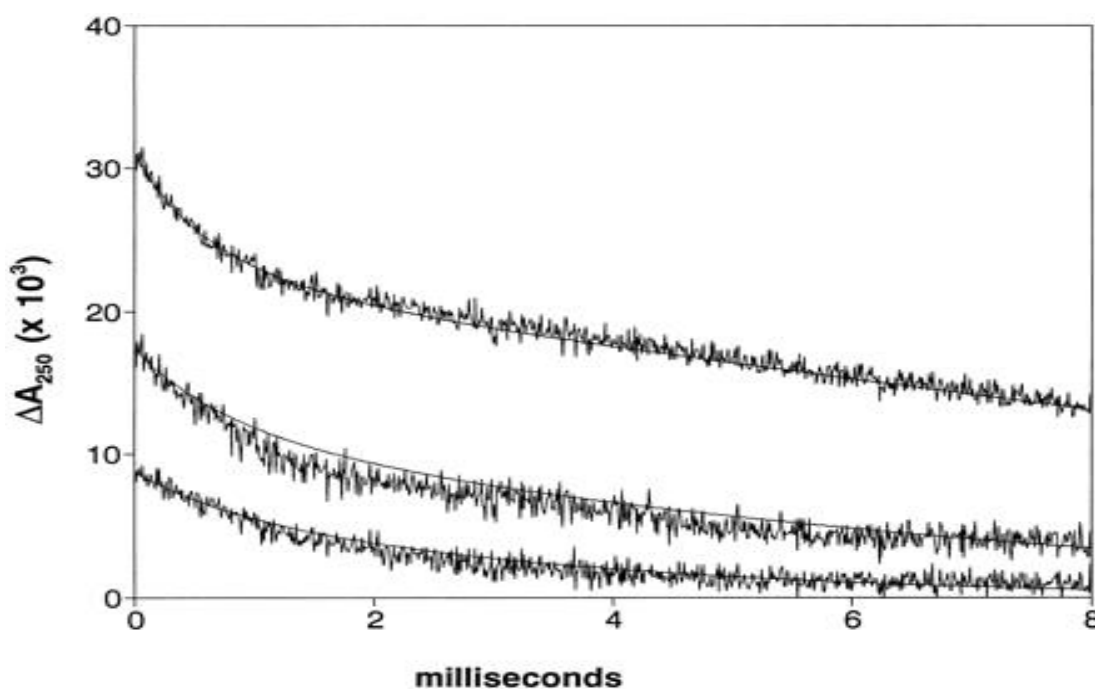


In the absence of other reactive components the radicals  $O_2^-$  and  $HO_2$  decay spontaneously by second order reactions as;



It was shown that at any given pH the rate of decay of the peroxy radicals was proportional to the concentration of the enzyme. The activity of superoxide dismutase following an electron pulse, by following decrease in absorbance at 235, 245, 270 and 300 nm show the same rate constant. It was

evident that the activity of superoxide dismutase is hardly affected by pH, in the range 4.8 – 9.5, but did diminish as the pH was lowered beyond 4.8. This observation and the fact that  $pK_{H_2O_2} = 4.8$ , suggest that the enzyme actually acted upon the superoxide anion rather than  $HO_2$  (Kempner, 1999).

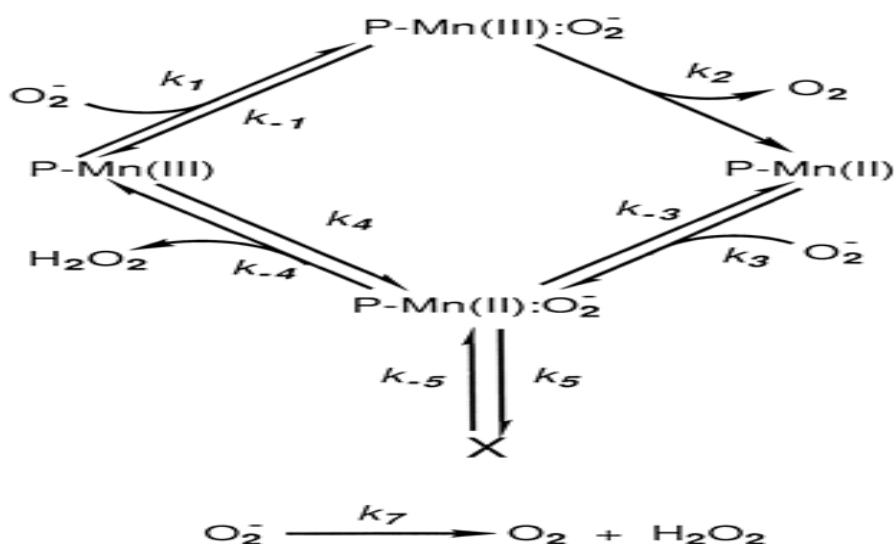


**Fig. 3. Superoxide decay catalyzed by human manganese superoxide dismutase as determined by pulse radiolysis.**

Data show the decrease in absorbance at 250 nm due to superoxide. Traces from the top of the figure down contained 11.6, 6.5, and 3.4  $\mu\text{M}$  as initial concentrations of superoxide. Kinetic study to determine the catalytic properties of human manganese superoxide dismutase, shows that the catalysis of the decay of  $O_2^-$  by human MnSOD has a biphasic pattern in which an initial first-order decay of  $O_2^-$  was quickly followed by an extended region of a much slower zero-order disappearance of  $O_2^-$ . This was observed by pulse radiolysis (Fig. 1) and is consistent with observations of previous kinetic studies of bacterial MnSODs (Robert et al., 1974).

### Mechanism

Model shown in Scheme I below is based on oxidative and reductive half-cycles of the accepted mechanism of the SODs and includes formation of a dead-end complex (X in Scheme I). Previous report (Bull et al., 1991) have reported spectroscopic evidence for the nature of this inhibited complex and suggest that it is a side-on peroxy complex with manganese (III). Active enzyme is regenerated by dissociation of this complex to the  $Mn^{+3}$  form and free peroxide, as shown in steps 4 and 5 of Scheme I. The regeneration of active enzyme, by this model, is a dominant factor in the zero-order region of the catalysis (Bull et al., 1991).



**Scheme I: Regeneration of active enzyme, by this model, is a dominant factor in the zero-order region of the catalysis (Bull *et al.*, 1991).**

The advantage of pulse radiolysis data compared to stopped-flow data is that they contain information from the initial burst of catalytic activity. It is apparent that the major difference in the catalytic behavior lies in the steps of the formation of the inhibited complex, step 5. In particular, the rate constant for formation of this complex  $k_5$  is 30-fold faster for human compared with the bacterial MnSOD. This rapid formation of inhibited complex with the human SOD would explain why we have not been able to observe even the tail end of the burst phase with human MnSOD using stopped-flow methods (Adam, 1979).

### Conclusion

The main application of pulse radiolysis is in the modification of amino acids side chains which lead to partial or complete inactivation of the enzyme involved. Studies carried out on superoxide dismutase using this technique includes identification of amino acids crucial to the enzyme activity, determination of the enzyme mechanism of action, regulation of enzyme activity and determination of the target size of the enzyme.

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