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Copper Zinc Superoxide Dismutase

by

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Abbreviations:
EPR (electron pair resonance)
SOD (superoxide dismutase)
CuZnSOD (copper-zinc superoxide dismutase)
k_{-1} (the reverse reaction rate constant for equation 1)
$Cu^{+}SOD$ (specifies CuZnSOD's valence state as +1)
$Cu^{2+}SOD$ (specifies CuZnSOD's valence state as +2)
ROS (reactive oxygen species)

Table of Contents

Abstract……..2

Introduction……2

Molecular Properties and Structure……3

Mechanism of Enzymatic Catalysis……5

Detection, Activity, and Thermodynamics…….7

Summary……10

References……..10

Abstract

Copper-zinc superdioxide dismutase is a highly stable celluar enzyme whose only known catalytic activity is in the transformation of superoxide radicals to less oxidative H_2O_2 and O_2. The structure of CuZnSOD and it's mechanism of action are the primary focuses of this review.

Introduction

Haemocuprein (bovine), hepatocuprein (horse), and erythrocyte protein (blood) were all copper containing proteins discovered between 1938 and 1969 when Fridovich and McCord discovered the enzymatic superoxide dismutase function of erythrocytes via pulse radiolysis [4]. Zinc was also a known component of erythrocytes at that time [4].

A discussion of the other major SOD known (MnSOD) is beyond the scope of this review, however, its existence (primarily in the mitochondria) and its functional homology to CuZnSOD is important to note.

CuZnSOD's are extremely stable, making their purification from tissue much easier than other enzymes [4]. They are present in almost all eukaryotic cells and within cells they are located in the cytosol, in some lysosomes, in some peroxisomes, in the nucleus, and in the space between the inner and outer mitochondrial membranes [4]. Prokaryotic cells also contain CuZnSOD [4].

The prescence of CuZnSOD in these locations is thought to be related to general and specific protection mechanisms against ROS.

Molecular Properties and Structure

Table 1 [9]. CuZnSOD from Bovine Erythrocytes

Property or Characteristic	Value
Molecular weight	31200 g/mol
Subunit compositions	2 identical subunits, 151 amino acid residues each, one intramolecular dissulfide bond per subunit
Metal ion content	One Cu2+ and one Zn2+ per subunit for native protein
Color	Native protein (blue-green), apoprotein (colorless)
Isoelectric point	4.95
Native Protein λ_{max} (nm) Native Protein ε_{max} (M^{-1} cm^{-1})	258 250-270 680 10,300 Fine structure 300
Redox potential in native protein	E° = 0.42 V (for pH 5-9.5)

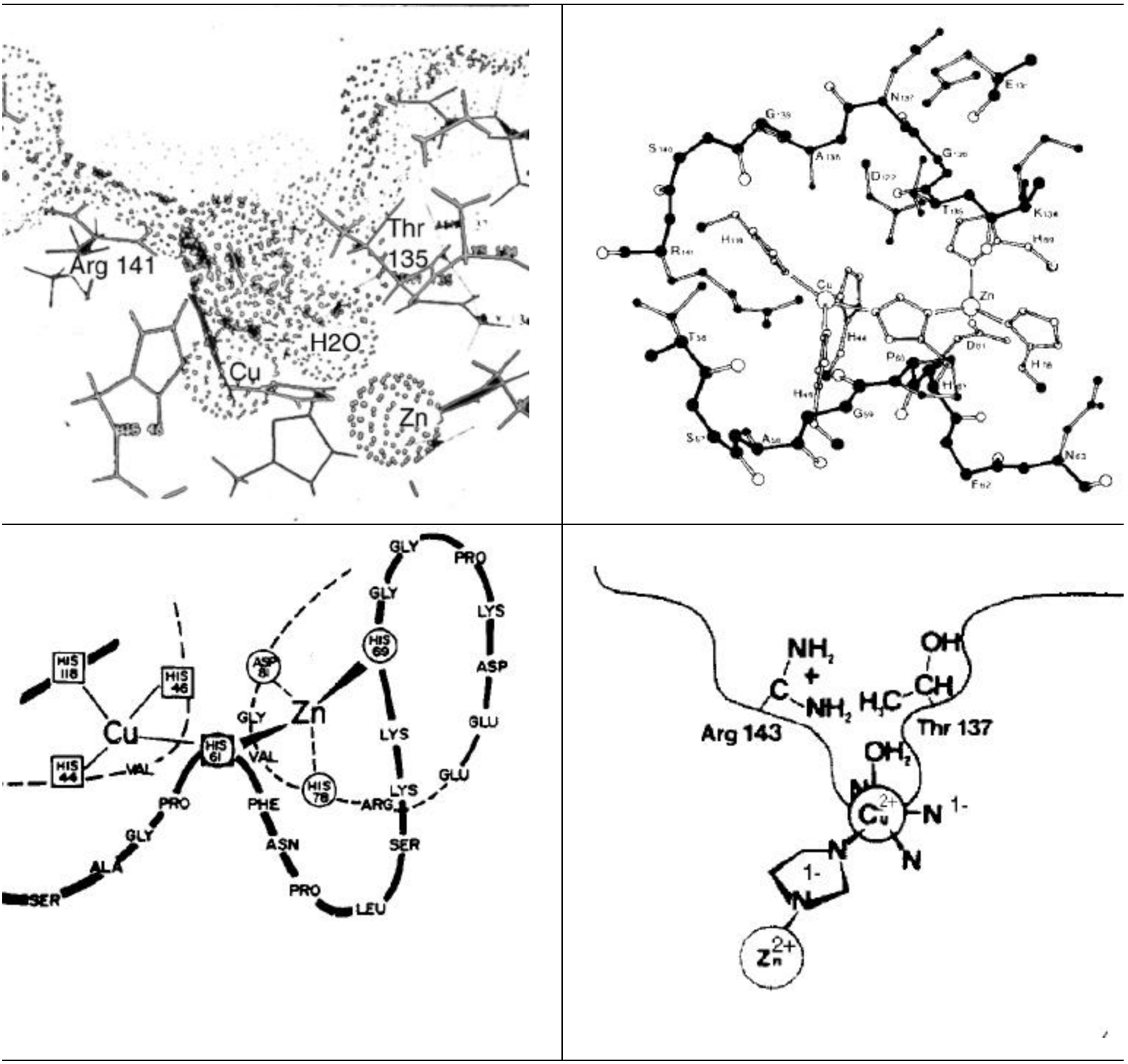

Figure 1. Four representations of CuZnSOD's active site are shown above. Each emphasizes different aspects of the same structure to allow for both a three dimensional

visualization and structural clarity. The enzyme as a whole is several times larger and usually occurs as a dimer [10]. The active sites of the two dimers are oriented like two funnels with the narrow ends connected, creating a line through both of their axis'. The inner surface of each active site is coated with a positive charge that sucks superoxide into the bottom of the funnel. Copper and zinc are connected by a His-61, a dinitrogen imidazole, in the active site. Arg 143 provides a hydrogen cation to a superoxide during the dismutation reaction.

Mechanism of Enzymatic Catalysis

The biochemical redox mechanism of copper in the CuZnSOD enzyme's active site protects cells from oxidative stress by removing superoxide from their internal milieu and replacing it with the less oxidative H_2O_2. The oxidative stress caused by hydrogen peroxide is then removed via catalase enzyme activity (4).

$$E\text{-}Cu^{2+} + O_2^{\bullet -} \leftrightarrow E\text{-}Cu^{+} + O_2 \quad (1)$$

$$E\text{-}Cu^{+} + O_2^{\bullet -} + 2H^{+} \rightarrow E\text{-}Cu^{2+} + H_2O_2 \quad (2)$$

$$2\,O_2^{\bullet -} + 2H^{+} \rightarrow H_2O_2 + O_2 \quad (3)$$

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (4)$$

$$H_2O_2 + Cu^{+} \rightarrow {}^{\bullet}OH + {}^{-}OH + Cu^{2+} \quad (5)$$

Superoxide's toxicity is also demonstrated through this pathway's reduction of copper (1) followed by the ping-pong oxidation of copper (2) and the later production of highly destructive hydroxyl radicals (5) [2]. Reaction (3) simply shows the combination of reactions (1) and (2).

This biochemical mechanism led to a larger enzymatic mechanism (Figure 2) [10]:

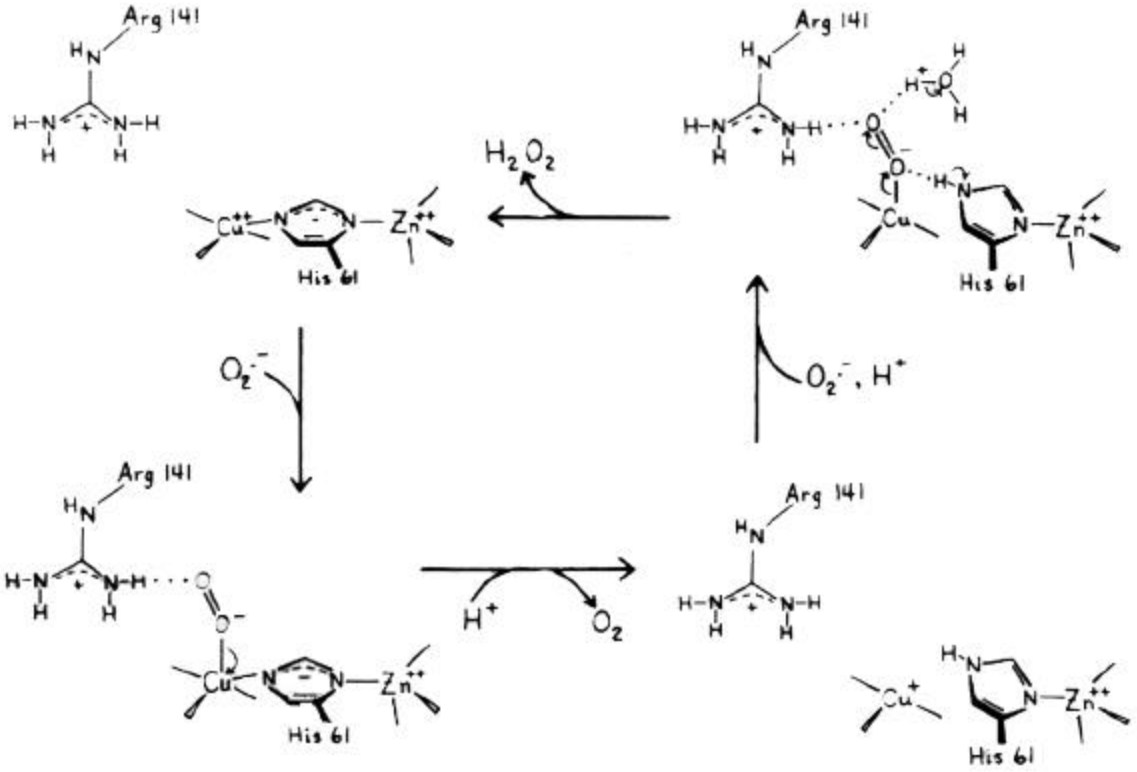

Figure 2. The CuZnSOD enzymatic mechanism begins in the upper left and proceeds counter clockwise. The high E-Cu^{2+} redox potential (0.42 V) as compared to aqueous Cu^{2+} (0.17V) enables the first electron transfere [10]. Histadine and Arginine provide delocalization of ionic charges during intermediates. His 61 also anchors zinc during the transformation. Zinc itself stabilizeds the enzyme without taking part in the catalysis [4].Cyanide and thiols inhibit the CZnSOD enzymatic mechanism [4].

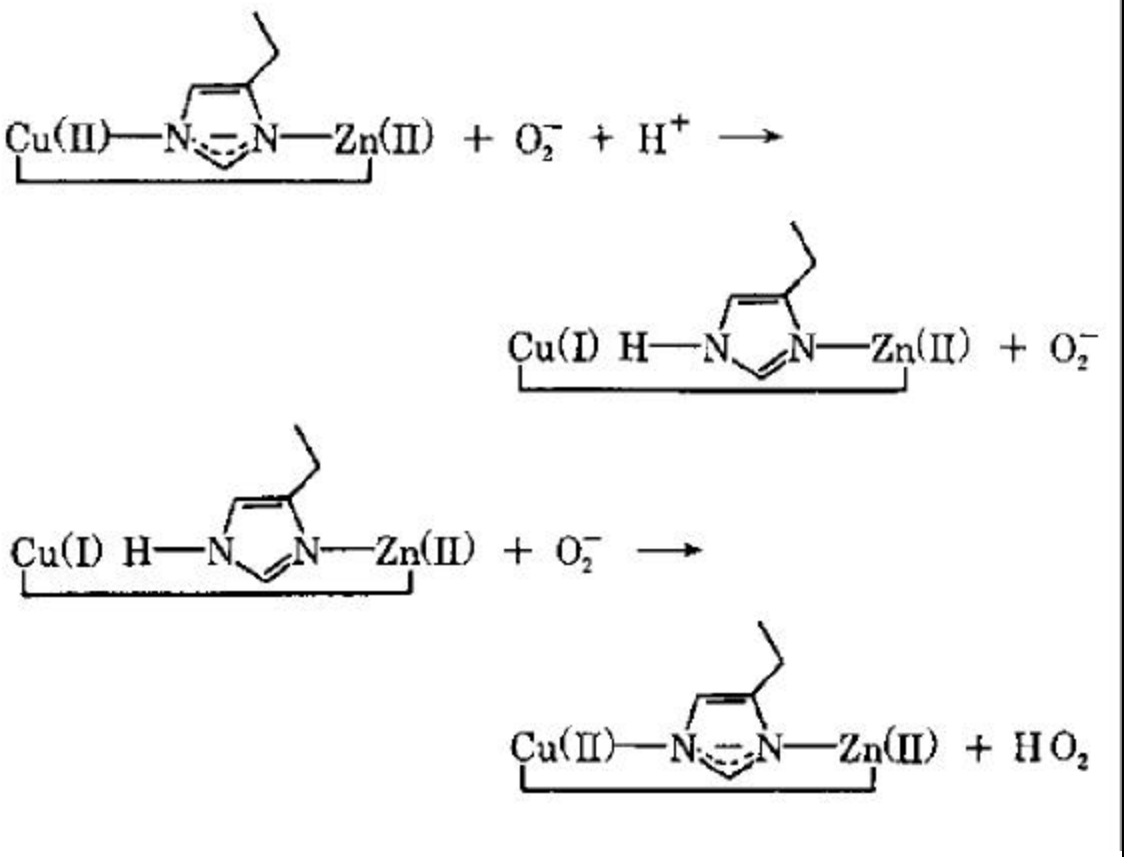

Figure 3. His 61, the imidazole between copper and zinc, is a proposed source of the hydrogen ions in (2) [11].

Detection, Activity, and Thermodynamics

ESR has been extensively utilized to investigate the structure of CuZnSOD and related compounds. The following EPR's are presented as examples of the type of spectra obtained from CuZnSOD variants [11]:

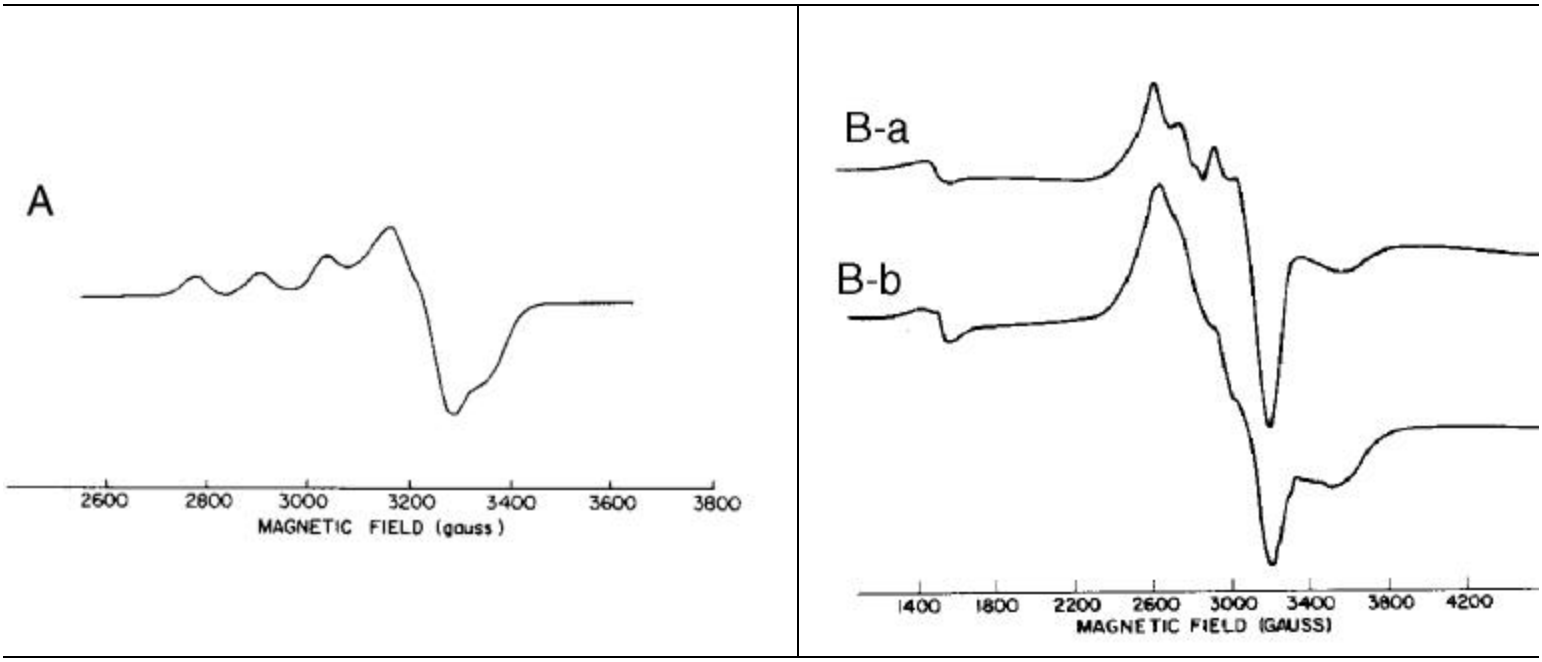

Figure 4. 77 K solutions are used to see (A) $Cu^{2+}Zn^{2+}$SOD, (B-a) a superimposition of zinc-free CuZnSOD at pH 9.3, and (B-b) $Cu^{2+}Cu^{2+}$SOD.

Pulse radiolysis is a direct form of CuZnSOD activity measurement, but it must be conducted with short observation times and pH's elevated far above the physiological range. *In vitro* pulse radiolysis experiments origionally showed little difference between the dismutation activity of CuZnSOD and other copper complexes found in cells [2]. Under oxygen species conditions applicable to those found in cells ($[O_2^{\bullet-}] = 10^{-11}$ M, $[O_2^{\bullet-}] < [O_2]$), the the ternary complexes formed between large biomolecules such as DNA and copper complexes such as $(salicylate)_2Cu(II)$ and $(tyrosine)_2Cu(II)$ indicate that the dismutation of superoxide occurs very slowly ($k_{-1} = 10^3 - 10^5$ $M^{-1}s^{-1}$ and $k_1 = 10^8 - 10^9$ $M^{-1}s^{-1}$) and is thought to be the reason why SOD's dismutation activity ($k_{-1} = .44$ $M^{-1}s^{-1}$ and $k_1 = 10^8 - 10^9$ $M^{-1}s^{-1}$) is so unique [2].

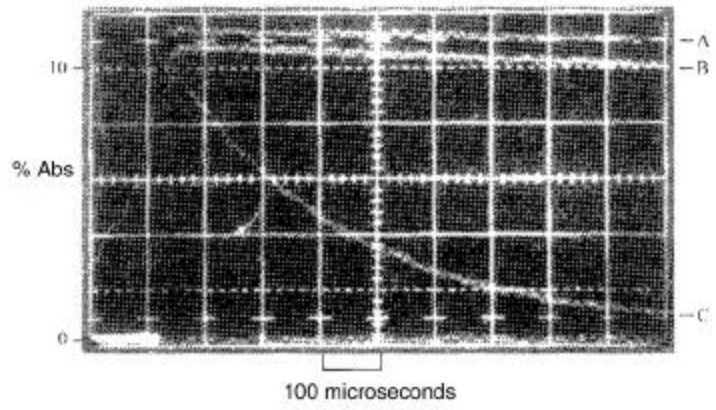

Figure 5. SOD catalysis is shown here with pulse radiolysis at pH 8.8 and initial [$O_2^{\bullet-}$] at32 micromole/liter. Measurements were made at 250 nm. Spontaneous decay (A), decay with 5 minute boiling inactivation (B), and decay with intact SOD. [SOD] = 2 micromoles/liter.

Other methods of detecting and analyzing CuZnSOD include the xanthene oxidase-cytochrome c assay, and photochemistry/spectrophotmetry [3].

The CuZnSOD compound is considered to be a thermodynamically poor catalyst when the ratio of Cu^{+}SOD to Cu^{2+}SOD is greater than 100 or less than .01; the minimum redox potential of an enzyme capable of dismutating superdioxide under physiologic conditions can be calculated with the Nernst equation using the following table's ratios, $E° = -0.16V$ for $O_2^{\bullet-}/O_2$, $[O_2] = 0.24mM$, and $[O_2^{\bullet-}] = 10^{-8}\ 10^{-11}$ M Quantitative [2]:

Table 2 [2].

	E° (mV) Cu+SOD / Cu2+SOD				
$[O_2^{\bullet-}]$ M	0.01	0.1	1.00	10	100
10^-11	157	216	275	334	393
10^-10	98	157	216	275	334
10^-9	39	98	157	216	275
10^-8	-20	39	98	157	216

So far the redox potentials of bioactive copper compounds other than CuZnSOD have not been high enough to be competitive with the values given in Table ? [2].

While the detection of CuZnSOD and its activity have been made possible through ESR and pulse radiolysis. Their usefulness is limited by the enzyme's environmental conditions and

should be kept in mind when creating an experimental design. The structural conditions of CuZnSOD's active site and concentrations of the dismutation elements control the thermodynamic effect exerted on the enzymatic system.

Summary

Copper-zinc superdioxide acts as a valuable catalytic superoxide detoxifier in mammalian cells. It has a well defined structure and mechanism of action. While many methods for detecting and analyzing CuZnSOD exist, methods for determining CuZnSOD's quantity and activity *in vivo* have not been found. CuZnSOD has a definite role in the regulation of oxidative stress and the knowledge gained from it's study will lead us to further discoveries concerning the major regulatory pathways of a process involved in most known mammalian diseases.

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