

Cu-Zn SUPEROXIDE DISMUTASE

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INTRODUCTION

Three distinct types of superoxide dismutase are known, each differing with respect to the metal at the active site (1). SOD (superoxide oxidoreductase, EC 1.15.1.1) is believed to be present in all oxygen metabolizing cells. Each type is distinguished by its amino acid sequence and each contains different prosthetic metals, Cu, Zn-, Fe-, and Mn-enzyme (2-5). This enzyme catalyzes the reaction



(6). There is considerable evidence that this enzyme is absolutely necessary for the survival of all oxygen-metabolizing cells (1).

SUPEROXIDE

The perhydroxyl radical (HO_2), the protonated form of the superoxide radical (O_2^-), was first postulated by Haber and Willstätter (7) to react with hydrogen peroxide



HO_2 could then reappear by reaction [3],



Reactions [2] and [3] were later referred to as the Haber-Weiss (8) cycle.

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The superoxide ion is both an oxidizing agent and a reducing agent with a reduction potential (E°) of 0.94 V for the O_2^-/H_2O_2 couple and -0.33 V for the O_2/O_2^- couple at pH 7 (9). Thus, the superoxide ion is thermodynamically unstable in water. In aprotic media the reduction potentials are -0.50 V for O_2/O_2^- and -1.75 V for O_2^-/HO_2^- (in dimethylsulfoxide) (10). Here superoxide is a mild reducing agent but a very weak oxidizing agent.

O_2^- is not especially reactive in that it will not react directly with alkanes and alkenes but it can act as a nucleophile (11-13). It can also reduce molecules such as cytochrome c, and oxidize molecules such as ascorbic acid (14). However, the presence of superoxide in biological systems can result in chaos if left unchecked. It is thought that a more damaging species is produced from superoxide, a likely candidate being the hydroxyl free radical. However, the rate constant for equation [3] appears to be much too slow for this reaction to be the source of hydroxyl radical (15). However, in the presence of metal ions it would appear that the hydroxyl free radical can readily be produced (16-18) by



Thus, it may be that the protective effect of superoxide dismutase may be in the minimization of reaction [5] and subsequently reaction [6].

SUPEROXIDE DISMUTASE

The Cu,Zn-superoxide dismutase had been isolated and studied for sometime (19,20) as a copper-containing protein of unknown function. In 1968-1969 McCord and Fridovich discovered that this protein, hemocuprein, catalyzed the dismutation of superoxide (6), reaction [4] above. In the absence of superoxide dismutase this reaction is exceedingly slow, if it occurs at all, $k < 1 M^{-1}s^{-1}$ (21). However, in the presence of SOD the second order rate constant has a value of $2.4 \times 10^9 M^{-1}s^{-1}$ (22) and as such is nearly a diffusion controlled reaction.

The Cu,Zn-SOD has been found in animals, fungi, plants and even in some primitive life forms. Animals have both the Cu,Zn- and Mn-enzymes. The Cu,Zn-SOD is usually

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considered to be associated with the cytosol and the intermembrane space whereas the Mn-SOD localizes in the matrix, including the intermembrane space of liver mitochondria (23). The Mn-enzyme has also been found in liver cytosol (23).

Cu,Zn-SOD STRUCTURE

Bovine Cu,Zn-SOD is a dimer of two identical 151-residue subunits, each containing one copper and one zinc atom (24). The X-ray crystallographic work shows that approximately 50% of the backbone is involved in a cylindrical barrel with the interior packed with hydrophobic side chains (25). Within each subunit the copper and zinc atoms are only about 6Å apart; however, the two copper atoms of opposite subunits are approximately 34Å apart. The zinc is in a tetrahedral arrangement and the copper has four histadines as ligands in an approximate plane with one side of the copper open for solvent access. The copper and zinc share a common histadine ligand.

It has been observed that the Arg 141 residue of bovine erthyrocyte SOD is essential for enzyme activity (26). This residue is within 6Å of the copper in the active sight and is also in the solvent access channel. The presence of this arginine increases the catalytic efficiency by approximately one order of magnitude. A plausible role for Arg 141 is that of facilitating proton conduction to produce HO_2^- as a leaving group in the catalytic cycle (26).

Koppenol has analyzed the charge distribution of the Cu,Zn-SOD (26a) and has determined that the protein has a small dipole moment, ~ 80 debye, and that the net charge and distribution of charges oppose the binding or a specific orientation of the protein to a negatively charged membrane. In addition, at pH 7, the net charge of the dimer is $-3e$. His electrostatic potential field

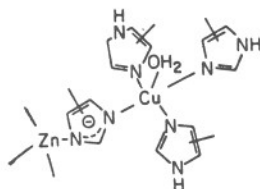


Figure 1. A representation of the active site of Cu,Zn-SOD.

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calculations show that the potential is such to repel O_2^- except near the active site. Neutralization of Arg 141 increases the electrostatic barrier. It seems as if superoxide dismutase is extremely well suited to assist in the dismutation of superoxide.

MECHANISM OF ACTION

The turnover of Cu,Zn-SOD is governed by a second-order rate constant of $2.4 \times 10^9 \text{ M}^{-1}\text{s}^{-1}$. The enzyme appears to function by a "ping pong" mechanism of having its copper alternately reduced by one superoxide ion and then reoxidized by a second (22,27). Thus, the copper cycles between an oxidation state of (II) and (I) whereas the iron and manganese-containing enzymes cycle between states (III) and (II). EPR studies suggest a low spin density on the Zn(II) ion (28) in the Cu,Zn-SOD, thus the role of the zinc has been relegated to a structural function. However, the charge the zinc ion brings to the protein may be the key to the correct electrostatic potential needed to direct the superoxide ion to the active site, as well as increasing the electrostatic attraction between the active site and O_2^- . It has been estimated that 10% of all collisions of Cu,Zn-SOD with O_2^- lead to a reaction, whereas 25% of all collisions of Cu(II)(aq) with O_2^- result in a reaction (26a). Cu,Zn-SOD is approximately 150 times larger than the hydrated copper ion. Thus, SOD is extremely effective in catalyzing the dismutation reaction (equation [4]).

O_2^- AND SOD IN HUMAN HEALTH

The discovery of the superoxide dismutase activity of hemocuprein has brought about the realization that the rather exotic superoxide ion is produced in living systems requiring oxygen. Superoxide and superoxide dismutase are implicated in inflammation (29), chronic granulomatous disease (30), phagocytosis (31), alloxan induced diabetes (32), 6-hydroxydopamine effects on catecholamine nerve terminals (33), radiation damage (34), paraquat toxicity (35), arthritis (36), muscular dystrophy (37), aging (38-39) and cancer (39-42). There is now evidence surfacing which suggests that the SOD's may have some other function in addition to their protective role (41). The evidence suggests that O_2^- , H_2O_2 and SOD are intimately involved in cell differentiation and cell division. This possibility necessitates

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a new direction for O_2^- -SOD research. There is now a report that SOD may be necessary for the establishment of the interferon-mediated antiviral state (43). Thus, additional functions for SOD are a real possibility.

The biological aspects of superoxide ion and superoxide dismutase still require much research for understanding. The impact of these endeavors are yet to be realized.

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DISCUSSION

Smith: Have you considered the matrix in which the SOD exists in the cystol? There should be no difficulty in obtaining a proton because the environment in which SOD operates contains orders of magnitude excess amino acids, proteins, and other proton sources like them. So what you are looking at is a very dilute species in a complex chemical matrix. However, iron(II) can only exist in that mixture in vanishingly small quantities I would expect.

Buettner: You are absolutely right. Dr. Todder at the Oak Ridge National Laboratory has approximated the amount of superoxide produced by an organism in a day and he has suggested that it takes one part in 10^9 of oxygen being converted to superoxide and escaping the defense mechanisms to essentially equal one rad of radiation per gram of tissue. So even in vanishingly small amounts, it does not take much to accumulate these levels when considering the flux of oxygen through a living organism.

Smith: I am not saying there is not a defense mechanism for superoxide anion. I am merely saying that suggestions based on in vitro experiments may not hold in vivo.

Winterhalter: I would like to add just one comment. Clinically, people that have been transfused for a long time such as thalassemics have very high tissue iron stores and those iron stores are diminished by giving desferrioxamine. The promotion of iron excretion with desferrioxamine is extremely slow and can be enhanced by giving ascorbate. If one gives ascorbate and deferoxamine simultaneously to a person that has a very high iron store it often proves toxic and the speculation is that through the giving of ascorbate iron(II) is generated and leads to the formation of hydroxyl radicals.

Beuttner: Do you know what the charge on a desferrioxamine iron complex might be?

Winterhalter: I presume that it would be negative but I have not calculated it.

Weser: Regarding the survival of superoxide in the cell, I suspect that the life time of superoxide in lipid membranes of cells is much greater than it is in aqueous systems. However, the copper-zinc SOD is most abundant in the cytosol. We have determined that the SOD content of membranes

DISCUSSION

is below 0.3% of the total. If superoxide reaches the surface of membranes then hydroxyl radicals might be formed. So one might anticipate a need for SOD close to the membranes but it is only found in the cytosol. However, water which is a good scavenger of superoxide may react with it. So it is debatable as to whether the function of SOD is solely as a scavenger of superoxide. It may have a role as a copper transporting protein similar to the dual role for ceruloplasmin, enzymatic and transport.

Regarding the suggestion of controversy concerning SOD activity of small molecular weight copper chelates, I do not really think that they are that controversial. It has been shown that copper chelates are SOD active.

Buettner: I did not mean to imply that none of them act as SOD's.

Weser: The mixed valence copper complex of penicillamine is very stable. It survives treatment with a 500 molar excess of EDTA. It is also a much more effective SOD-like model compound. The mixed valence copper complex is a perfect SOD-mimetic model. However, one must use the correct penicillamine complex. If the incorrect complex is used then one may run into serious problems.

Buettner: I did not go into that because I assumed we would hear from you about that in much greater detail tomorrow.