



A REVIEW ON THE DESTINY OF SUPEROXIDE ANION RADICAL IN THE LIVING SYSTEM

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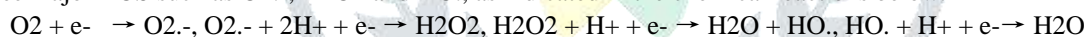
Abstract : Extensive studies have been conducted for many years on the oxidative stress in the living system and its correlation to the production and degradation of reactive oxygen species (ROS). Protection against oxidative damage due to superoxide radical in aerobic organisms became evident to the scientific community, after the discovery of the function of superoxide dismutase- a ubiquitous enzyme by Fridovich in 1969. Subsequent studies have shown that the aerobic organisms have evolved four independent enzymes for harvesting superoxide dismutation. They are FeSOD, MnSOD, Cu-Zn SOD and NiSOD. Detoxification of superoxide radicals in anaerobic organisms is by a family of bacterial metalloenzyme called superoxide reductase (SOR). A comparative study of superoxide dismutase and superoxide reductase is being carried out in the review.

Abbreviations: SOD- Superoxide dismutase, SOR- superoxide reductase. ROS-Reactive oxygen species.

Key Words: Superoxide dismutase, Superoxide reductase.

I. INTRODUCTION

Dioxygen is a powerful 4 electron oxidizing agent, the reduction of which in 4 subsequent one electron steps, results in three major ROS such as O_2^- , H_2O_2 and $HO\cdot$, as indicated in the chemical reactions below.



Each one of these products are very reactive oxidizing agents which is harmful to the living cells and the organisms are constantly under the oxidative stress.

Superoxide anion radical (O_2^-) is the single electron reduction product of dioxygen one of the most abundant radicals produced in the biological systems that come in contact with air. This can act as a signalling agent, a toxic species or a harmless intermediate that decomposes quickly depending on the type of organism. Elevated concentration of this, along with other ROS (reactive oxygen species) in the living cells will result in the oxidative damage and aging. To protect against the growing levels of oxidative damage, antioxidant enzymes were evolved. Two such types of enzymes are SOR and SOD. In bacteria the production of superoxide is by the reduction of dioxygen by the electron transport chain (1,2), by flavin containing enzymes, by univalent redox reaction such as ascorbates, thiols etc..and by photochemical reaction (3). In higher organisms the major source of superoxide is in the mitochondria, where the oxygen is reduced by the electron transport chain. (4,5,6). Yet another source is the reduction by NADPH oxidase in phagocytic cells which helps in killing small microbes (7,8) which is an extracellular process. Because of the negative charge it is poorly diffusible through cell membranes and effectively constrained to intra- or extracellular locations, depending on its site of origin. Production of superoxide in the cytoplasm is an unanswered question. Superoxide can be toxic under physiological conditions despite its limited production, short half-life, and limited reactivity. Superoxide so produced can propagate the radical chain reactions, by reacting with small molecules like polyphenols, catecholamines, ascorbate and thiols (5,9). It can inactivate enzymes like catalase, glutathione peroxidase, aconitase. It is also a precursor for more potent oxidant like H_2O_2 . It can readily be converted into hydrogen peroxide ($k \approx 2 \times 10^5 M^{-1} s^{-1}$) and, in the presence of appropriate metal catalysts, produce hydroxyl radical via the Haber-Weiss reaction (10, 11). The hydroxyl radical so formed in the living system will react immediately with whatever biological molecule in their vicinity, producing secondary radicals of variable reactivity. As a result, intracellular defences against superoxide-mediated damage are robust (10, 12).

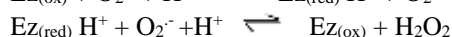
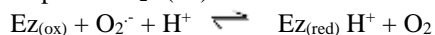
Biology has evolved two different enzymes such as superoxide dismutase and superoxide reductase in aerobic and anaerobic organisms respectively to defend against the superoxide mediate damage.

1.1 Superoxide dismutase:

Superoxide can undergo spontaneous dismutation reaction with a rate constant of $4 * 10^{-5} M^{-1} s^{-1}$ at a pH of 7.4. Since the intracellular concentration is about $10^{-10} M$, the half -life for dismutation is 3.5 hours. The need for a defense mechanism is

very much evident from these data (6). SOD's ubiquitous among aerobic organisms, provide a defense against oxidative stress by catalyzing the dismutation of superoxide into oxygen and hydrogen peroxide. This is a simple reaction incorporating the central theme of redox catalysis. The overall reaction

$2\text{O}_2^{\cdot-} + 2\text{H}^+ \rightleftharpoons \text{O}_2 + \text{H}_2\text{O}_2$ is brought about by Fe, Mn, Cu-Zn and Ni SODs in two steps which are both first order with respect to $\text{O}_2^{\cdot-}$ (10).



where the Ez(ox) and Ez(red) represent the oxidized and reduced state of the enzyme. The above-mentioned mechanism is common to all SOD's. Acquisition of a redox couple proton is indicated by the appended (H+). Proton uptake in conjunction with enzyme reduction has been presumed but not experimentally demonstrated for NiSOD. The first half of the reaction is favorable when the substrate gets oxidized. This is coupled with the acceptance of a proton by the enzyme in the reduced state, which facilitate substrate binding and reduction in the second half of the reaction. The overall disproportionation reaction involves the interaction of two superoxide anion radical crossing the electrostatic repulsion barrier. SODs overcome this difficulty by reacting with only one molecule of superoxide at a time, and the active site has the specificity towards binding of negative charge rather than the products which are neutral. The affinity towards negative charge is because of the net positive electrostatic potential at the active site due to the metal center, which is not fully neutralized by the surrounding ligands. This net positive charge not only favors the binding of substrate, but also a series of other small anions. The change in charge of the substrate while converting to the product favors the mechanism of substrate binding. The Ping-Pong mechanism suggested for SOD requires the binding of the substrate to two different versions of the same active site. It is especially interesting to note that, in the second half of the reaction, the anion has to bind to the reduced state of the metal center, having a lesser positive electrostatic potential. But this decrease in charge is compensated by the uptake of a proton. The net charge remains the same even though the charge distribution is different and the substrate binding should still be favored to a similar extent, if in a different mode. Oxidation of superoxide in the first half of the reaction given above, requires only the substrate binding and electron transfer, and it is favorable. Reduction of superoxide in the second half of the reaction is inherently unfavorable due to the transfer of electron to the antibonding π^* orbital. This was made possible by supplying a proton, required for the formation of H_2O_2 , from the enzyme. The favorable first half reaction is couple to the uptake of a proton which in turn can facilitate substrate binding and reduction. It is estimated that the superoxide reacts with SOD with a rate constant which is very close to the diffusion limit ($2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ at a pH of 7.4.) (6). This clearly shows the cellular defense exhibited by SOD.

1.2 Superoxide Reductase:

In the case of oxygen sensitive anaerobic organisms (methanogens, sulphate reducing bacteria and microaerophilic bacteria which survive under low partial pressures of oxygen) lack the antioxidant enzyme like SOD and catalase. The isolation and discovery of superoxide reductase SOR (13,14) has paved the way for understanding the defense against the oxidative stress in anaerobic organisms. They catalyze only one of the two reactions of SOD namely the reduction of superoxide to hydrogen peroxide which is self-evident from the name it bears.



Here the Rd_{red} is the rubredoxin in the reduced state, which is a one Fe:S metalloprotein acting as a competent electron donor. (15, 16). The reaction between SOR and superoxide is a second order with a rate constant of the order of $10^9 \text{ M}^{-1} \text{ s}^{-1}$ (17). However, there are questions remain about the reaction mechanism and the characterization of intermediates. Several research groups are trying to resolve this issue by using the mimicking models of the enzyme (18). Recently it was shown that Cu-Zn SOD could also act as SOR (19), which is very surprising. From this it is clear that the destiny of superoxide to dismutate, reduce or oxidize depends upon a variety of factors such as, concentration of the superoxide, enzyme and alternate redox species. The rate constant also plays a major role.

2. Conclusions:

Aerobic and anaerobic organisms have developed different strategies to fight against the oxidative stress due to superoxide, using two different enzymes, which are structurally and biologically different, but the same function. It is also very interesting to note the two detoxification path ways, from the point of view of the products formed and the potential advantages of each of these methods. In the case of SOD, two moles of superoxide can generate one mole each of O_2 and H_2O_2 , whereas the reaction catalyzed by SOR generates one mole of H_2O_2 per one mole of superoxide. Thus, the aerobic organisms prefer to have SOD whereas anaerobic and oxygen sensitive organisms prefer to have second pathway. The relative toxicity of H_2O_2 and O_2 in anaerobic organisms is yet another factor to be taken into consideration. O_2 can readily inactivate some enzymes involved in anaerobic fermentation pathway whereas H_2O_2 is a strong oxidant. Since these organisms does not have catalase enzyme, they have to find alternate detoxification pathway to remove H_2O_2 . Thermodynamically, both SOD and SOR has the reduction potential to oxidize and reduce the superoxide anion. But it is generally found that the SORs have SOD activity about three orders of magnitude lower than the SOD is still to be explored in future. The scientific community is looking forward with great interest to future surprises as this fascinating story of O_2 on earth continues to open out.

3. Acknowledgements:

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4. References:

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