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Superoxide Dismutase in Redox Biology: The roles of superoxide and hydrogen peroxide

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Abstract

Superoxide dismutases (SOD) are considered to be antioxidant enzymes. This view came about because its substrate, superoxide, is a free radical; in the era of their discovery, 1960's – 1970's, the general mindset was that free radicals in biology must be damaging. Indeed SOD blunts the cascade of oxidations initiated by superoxide. However in the late 1970's it was observed that cancer cells that have low activity of the mitochondrial form of SOD, MnSOD, grow faster than those with higher activities of MnSOD. These observations indicated that SOD, superoxide, and hydrogen peroxide affected the basic biology of cells and tissues, not just *via* damaging oxidation reactions. It is now realized that superoxide and hydrogen peroxide are essential for normal cellular and organism function. MnSOD appears to be a central player in the redox biology of cells and tissues.

Keywords

superoxide dismutase; mitochondria; coenzyme Q; hydrogen peroxide; superoxide; redox environment; hypoxia inducible factor; iron

Introduction

With the discovery of: 1) the superoxide dismutases by McCord and Fridovich in 1968–1969 [1]; 2) the concomitant discovery that superoxide can be produced by enzymes [2]; and 3) that free radicals can be substrates for an enzyme completely revolutionized the thinking on the role of free radicals and related oxidants in biology [3]. Because free radicals can bring about harmful oxidations, research initially concentrated on the chemistry of the individual reactive oxygen species and the damage they could inflict. In those early years much energy was invested in determining the kinetics and mechanisms of these detrimental oxidative reactions [4, 5, 6, 7, 8, 9].

In the 1980's the thinking in this new field was broadened with the introduction of a new, and then controversial, idea that at lower concentrations, superoxide or hydrogen peroxide could induce cell division [10, 11, 12]. This concept arose because it was observed that tumor cells have lower manganese superoxide dismutase (MnSOD) than corresponding normal cells, suggesting a role for superoxide in cell proliferation [10]. Most convincing of a role for SOD and superoxide was a study by Bize and Oberley on the Morris hepatomas [13]. In this tumor model, they found that the growth rate of the tumor was inversely proportional to the activity of MnSOD, Fig. (1). The idea that superoxide and hydrogen peroxide are important components of normal biology began to take firm hold in the 1990s

with the development of molecular biology techniques that allowed controlled expression of proteins; these new tools revealed that increased expression of manganese superoxide dismutase suppresses the malignant phenotype of human melanoma cells [14]. At this time the idea that ROS could be signaling molecules gained broader acceptance and the field of redox biology began to grow exponentially [15, 16, 17, 18]. Redox biology is now viewed by many as a fundamental determinant of the basic biology of cells and tissues, disease and health [19, 20]. SOD serves not only as a protective enzyme, but also has a central role in determining the basic biology of cells and tissues [21].

The flow of electrons

The major flow of electrons in normal cell metabolism is through the mitochondrial electron transport chain (ETC). There, oxygen is reduced to water producing energy that is harvested in ATP to build cellular structures and maintain them. A small percentage ($\approx 1\%$ or less [22, 23, 24]) is diverted *via* a one-electron reduction of dioxygen to produce the superoxide radical. These two pathways have been proposed as two distinct types of mitochondrial redox circuits [20]; a high flux circuit that results in energy production and a low flux circuit that results in regulation of metabolism and that initiates cell signaling, Fig. (2). The low flux circuit that produces $O_2^{\bullet-}$ and H_2O_2 appears to be controlled in part by superoxide dismutase (SOD) [21].

SOD and the peroxide-removing system assure that the steady-state levels of superoxide and H_2O_2 remain relatively low, Fig. (3). In addition to the electron transport chain, superoxide can be produced by the Nox-family of enzymes as well as xanthine oxidase [25]. H_2O_2 can be produced directly by several enzymes, for example oxidase enzymes in the peroxisomes [26, 27]. As signaling molecules, $O_2^{\bullet-}$ and H_2O_2 , have quite different chemistry; superoxide can be either a one-electron oxidizing agent, mostly via $HO_2^{\bullet}$, or a one-electron reducing agent; H_2O_2 is principally a two-electron oxidizing agent. These two different chemistries assure versatility as well as specificity in cellular signaling.

One-electron reactions: radicals and antioxidants

Most chemical reactions in living systems that involve organic molecules are two-electron reactions. In general one-electron reactions are avoided because they can result in damaging free radical processes. The damaging free radicals can be removed by one-electron reactions with small molecule antioxidants [28]. For example, in free radical-mediated lipid peroxidation, one-electron oxidants generate a carbon-centered radical on the lipid chain, Fig. (4). This radical reacts rapidly with oxygen forming reactive peroxy radicals. Donor antioxidants such as tocopherol can convert lipid peroxy radicals ($LOO^{\bullet}$) to a less reactive lipid hydroperoxide ($LOOH$). The tocopheroxy radical can be recycled in a one-electron reaction by ascorbate. The ascorbate radical is quite unreactive and thus far less damaging than the original peroxy radical, Fig. (5).

This sequence of reactions has two major consequences:

1. The dangerous one-electron oxidant is exported from the vulnerable lipid phase into the aqueous phase of cells where enzyme systems can detoxify and “recycle” ascorbate.
2. The remaining oxidant in the lipid phase ($LOOH$) is now available to the 2-electron antioxidant system for use or removal.

One-electron reactions, redox active metals and electron flux

One-electron reactions are common when redox active metal ions such as copper, manganese, or iron are involved. These metals are essential parts of the active sites of many enzymes, including the superoxide dismutases. Superoxide can be an oxidant, a reductant, or both, depending on the coordination environment of the metal. The production of superoxide by the mitochondrial electron transport chain is the gateway to the low-flux signaling circuits of cells and tissues, Fig. (2); it provides the connection to the high flux, energy-harvesting circuits of ATP production. Superoxide reacts with the metal-containing active site of SODs alternately as a reductant and then as an oxidant, yielding oxygen and H_2O_2 . The steady-state level of $\text{O}_2^{\bullet-}$ is inversely related to [SOD] [21]. Thus, SOD controls the flux of electrons entering the one-electron signaling and regulatory pathways, Fig. (2). Hydrogen peroxide is a product of the SOD-catalyzed dismutation of $\text{O}_2^{\bullet-}$. This H_2O_2 is a key regulator of the redox states of the various redox couples of two-electron signaling circuits. Thus, in the mitochondria, superoxide is the entry point for both the one-electron and two-electron signaling circuits and MnSOD is at the node of the bifurcation point for these pathways.

One-electron reactions, redox active metals and gene expression

Superoxide can influence gene expression by one-electron reactions. It has recently been shown that superoxide, *via* manganese-containing superoxide dismutase (MnSOD), can regulate the levels of the transcription factor hypoxia inducible factor-1 α (HIF-1 α) [29, 30]. The HIF-system is responsible for sensing the local levels of oxygen and regulates literally hundreds of genes ranging from basic metabolism, angiogenesis, to cell proliferation [31]. There are many routes to the modulation of the levels of HIF-1 α ; the primary route centers on the hydroxylation of HIF-1 α by prolyl hydroxylase (PHD), Fig. (6). When oxygen is present, PHD can hydroxylate HIF-1 α marking it for degradation [31]. However, when the levels of dioxygen are low, HIF-1 α accumulates and moves to the nucleus where it assembles into the transcription factor HIF-1.

PHD is an iron-containing enzyme; the sensing of oxygen as well as the hydroxylation reaction are all centered on iron. This iron has a very loose coordination environment, with two histidines and a carboxylate group (Asp or Glu) from the PHD protein as ligands, Fig. (7). The ligands for the remaining three coordination sites, α -keto-glutarate and dioxygen, are transient. During the catalytic reaction, molecular oxygen is fully reduced; two electrons come from α -keto-glutarate, which leaves as succinate and CO_2 , the remaining two electrons come from a proline. The coordination environment of the Fe^{2+} in PHD would be amenable to reaction with $\text{O}_2^{\bullet-}$ (oxidation *via* $\text{HO}_2^{\bullet}$). In addition the ferryl (*i.e.* Fe(IV)) intermediate that is formed during the catalytic cycle [32] would be especially vulnerable to a reducing agent such as superoxide. In both of these possible reactions of superoxide, the Fe^{2+} of the resting state enzyme and the ferryl intermediate generated in the catalytic cycle would be converted to Fe^{3+} ; the result would be the inactivation of PHD and subsequent accumulation of HIF-1 α . Thus, for example, decreasing the activity of MnSOD in mitochondria could inactivate PHD, leading to increased HIF and subsequent gene expression [29, 30].

This example shows how one-electron reactions *via* superoxide with the iron of PHD-type enzymes initiates signaling cascades that result in gene expression, Fig. (2).

One-electron reactions, redox active metals and metabolism

The mitochondrial enzyme aconitase is a key enzyme in the Krebs cycle and thus is a fundamental link in the production of ATP. The active site of aconitase contains an iron sulfur cluster. Superoxide reacts with this cluster rapidly, $k \approx 10^7 \text{ M}^{-1} \text{ s}^{-1}$ [33, 34]. In this reaction superoxide actually oxidizes the Fe_4S_4 cluster of aconitase. There are amino acid residues near the active site that provide hydrogen bonding for the substrate, *e.g.* isocitrate, during the reaction [35]. These residues could also serve to protonate superoxide, facilitating the oxidation of the cluster. This oxidative reaction of superoxide inactivates the enzyme thereby slowing the basic energy metabolism of the cell. This inactivation provides a feedback mechanism from the low flux signaling circuit to the high flux circuit of energy metabolism, Fig. (2). Superoxide can be viewed as a brake for aconitase and ATP production. This is a good example of $\text{O}_2^{\bullet-}$ being an efficient effector-molecule in controlling metabolic switches. The inhibition of aconitase has additional ramification. Its inactivation would lower the production of α -ketoglutarate, which could lead to accumulation of HIF-1 α because α -ketoglutarate is an essential cofactor of PHD.

The one-electron, low-flux circuits originating in mitochondria provide feedback to the high flux circuit of ATP production. In addition these low flux circuits connect to metabolism and gene expression. The H_2O_2 produced from superoxide is an entry point to two-electron signaling processes.

Two-electron signaling

The low flux, two-electron circuitry has NADPH and thiols as fundamental redox couples. The conversion of hydrogen peroxide to water requires two reducing equivalents. The glutathione peroxidase family of enzymes (GPx) uses glutathione as this source of reducing equivalents.

The peroxiredoxin (Prx) family of enzymes also removes H_2O_2 with thioredoxin as the principal source of reducing equivalents.

Each system gathers electrons from NADPH to return GSSG and Trx(SS) back to their corresponding reduced forms, GSH and Trx(SH)₂, Fig. (3).

Changes in the flux of H_2O_2 will present differing demands on these reducing systems and thereby change the redox state of these redox couples; the flux of H_2O_2 would contribute to the redox state of the $\text{GSSG}, 2\text{H}^+/2\text{GSH}$ couple, the principal component of the redox buffer of cells and tissues [19, 20]. Changes in the redox state of these couples will have consequences on signaling cascades and thereby on the biological state.

Two-electron reactions: redox buffer and enzyme activity

Cells and tissues must maintain a reducing environment to survive. The reducing environment is maintained by a redox buffer that consists of an array of linked redox couples, Fig. (3). The redox environment of a cell is a reflection of the redox state of these

couples [19]. Glutathione is considered to be the major component of this redox buffer. H_2O_2 produced by the mitochondrial low flux circuit, *via* superoxide and MnSOD, can change the state of the redox buffer by drawing on the reducing equivalents of the buffer [36]. The flux of H_2O_2 can change the status of the GSSG/2GSH couple to a more oxidized state as H_2O_2 is reduced to water by GPx. The metabolic flux of H_2O_2 and glutathione disulfide reductases can keep the GSSG/2GSH buffer within its normal range for the appropriate biological state. However, if the flux of H_2O_2 is increased, then GSSG may accumulate to higher levels; this can alter the redox state of the GSSG/2GSH couple and potentially change the biological state of the cell, Fig. (8).

In general, cancer cells are considered to be under oxidative stress. However, there is good evidence that the redox state of the intracellular GSSG/2GSH couple remains relatively reduced ($E_{\text{GSSG}/2\text{GSH}} \approx -240 \text{ mV}$); this reduced status correlates with proliferation [19]. The intracellular redox state of the GSSG/2GSH couple is more positive in cells that are not proliferating. Changes in the redox state of the intracellular redox buffer are associated with the cell cycle; as initially proposed by Goswami *et al.*, there is a redox cycle within the cell cycle and MnSOD appears to be fundamental element in this redox cycle [37, 38, 39].

Although oxidative stress is often thought of as producing damage, a better view is that oxidative stress disrupts cellular redox circuits [20]. This disruption would occur when there is an imbalance of oxidant production and removal. MnSOD in the mitochondria would be a key player in establishing the appropriate balance in the redox circuitry of mitochondria.

MnSOD, the bifurcation point between 1- e^- and 2- e^- signaling

Superoxide dismutase has traditionally been considered as an antioxidant enzyme. However, as presented above, it is clear that MnSOD also is central in setting the basic biology of cells. It is clear that the steady-state level superoxide is inversely proportional to the activity of MnSOD [21]. However, SOD can also influence the flux of $\text{O}_2^{\bullet-}$ and consequently the flux of H_2O_2 in some settings. SOD will not affect the flux of $\text{O}_2^{\bullet-}$ or H_2O_2 from typical sources in which flavins are the conduit for electrons to dioxygen, such as xanthine oxidase. However, SOD will be able to alter the flux of $\text{O}_2^{\bullet-}$ or H_2O_2 from some quinone/semiquinone/hydroquinone triads, providing the thermodynamics and kinetics are appropriate [40]. The coenzyme Q triad appears to have the appropriate thermodynamics and kinetics for SOD to “pull” the reaction of coenzyme Q semiquinone with dioxygen to form superoxide [21],

An increase in the levels of H_2O_2 with increasing activity of SOD in cells has been observed [21, 41, 42, 43, 44]. These reactions allow SOD to affect both the 1-electron and 2-electron signaling pathways, *i.e.* MnSOD is at the bifurcation point between these two separate signaling pathways Fig. (9). These possibilities explain in part how changing the activities and ratios of SOD and GPx in tumor cells can affect cell doubling time and tumorigenicity [41, 45, 46]. This source of H_2O_2 also appears to have a major role in metastases, reviewed in [47]. Thus, it is clear that MnSOD is a central redox enzyme, *i.e.* it plays a major role in establishing the biology of cells and tissues.

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Figure 1.

The rate of tumor growth is inversely related to SOD activity. Shown are total SOD activity in slow-, medium- and fast-growing Morris hepatomas. Data from [13].

Figure 2. The mitochondrial electron transport chain has two principal circuits

During the oxidation of carbon-based substrates, the vast majority of the electrons captured are shuttled to dioxygen to produce water, the high flux circuit; the energy is captured to produce ATP. In the low flux circuit a small fraction of the total electron flow moves to dioxygen in a one-electron reduction to produce superoxide. This superoxide can react with iron-sulfur centers such as aconitase to modulate the flux in the high flux circuit. Superoxide could also react with the iron-center of enzymes, such as the prolyl hydroxylases, inactivating the enzyme leading to accumulation of HIF-1 α and subsequent gene expression. MnSOD will control the steady-state level of mitochondrially-produced superoxide as well as the flux of H_2O_2 [21]. This will have effects on two-electron signaling pathways, the redox buffer and the biological state of the cell.

Figure 3. Overview of the thiol redox couples that set the redox status of cells and tissues
 SOD is central as it controls the steady-state level of superoxide as well as the flux of H_2O_2 .
 The flux of H_2O_2 affects both nodes of the peroxide removal/redox system, *i.e.* both the GSH and Trx nodes.

Figure 4. Shown is an overview of lipid peroxidation and points where tocopherol can act as a donor antioxidant

In this example iron-derived oxidants are shown as initiators. In addition Fe^{2+} is shown as a facilitator of chain-branching reactions. Although lipid-derived alkoxyl radicals ($\text{LO}^{\bullet}$) are quite oxidizing and could initiate new chains, *i.e.* $\text{LO}^{\bullet} + \text{LH} \rightarrow \text{LOH} + \text{L}^{\bullet}$, rearrangement to an epoxide is the dominant reaction [29, 30]. Shown also is an example of how two-electron processes remove LOOH , here phospholipid hydroperoxide glutathione peroxidase (GPx4) using GSH as a source of reducing equivalents.

Figure 5. Ascorbate is the terminal, water-soluble, small-molecule antioxidant [48]

Among its many functions as a reducing agent, ascorbate can serve as a one-electron reductant to recycle tocopherol. In this way it exports free radicals that are in a lipid structures (here $\text{LOO}^\bullet$ and $\text{TO}^\bullet$) to the aqueous phase where enzymes and other reducing agents can recycle $\text{Asc}^{\bullet-}$ back to AscH^- . By reducing $\text{TO}^\bullet$ (vitamin-E $^\bullet$) back to TOH further oxidation to tocopherol quinone is prevented; tocopherol quinone will form irreversible products and thus vitamin E lost. In addition it prevents $\text{TO}^\bullet$ from initiating further oxidations [49].

Figure 6. Prolyl hydroxylases serve as sensors of cell/tissue dioxygen controlling HIF-1α accumulation and subsequent gene expression

PHD is an iron-containing enzyme; the iron must be present as Fe²⁺. This ferrous iron activates dioxygen resulting in the oxidation of proline residues, leading to degradation. A lack of oxygen or disturbance of this iron will lead to accumulation of HIF-1α and subsequent gene expression. This simplified scheme does not show all the routes to regulation of HIF system.

Figure 7. The coordination environment of typical prolyl hydroxylases

Only three of the six coordination sites are from the protein. The remaining three sites are open so substrates, 2-oxoglutarate (2OG) and dioxygen, can “come and go”. This results in the iron being susceptible to oxidants, *e.g.* superoxide. (Protonation of superoxide yields $\text{HO}_2^\bullet$, a strong oxidant.) Protons from ionizable amino acids near the active site can facilitate this change in reactive character of superoxide. This type of structural feature and mechanism converts superoxide, a weak reductant, to $\text{HO}_2^\bullet$ an oxidant in SOD [50, 51].

Figure 8. Reduction potential-driven nano-switches move cells through different biological stages [19, 52]

The intracellular redox environment changes throughout its life cycle. For example, cancer cells have disruptions in the low-flux and high flux circuits that result in their not progressing to the quiescent or differentiated state. Rather they retain a more reduced intracellular environment and remain the proliferative state.

(A) During proliferation the half-cell reduction potential (E_{hc}) for the GSSG, $2H^+/2GSH$ couple has the most negative value. The switches for proliferation are fully on.

(B) When E_{hc} (GSH) becomes more positive, the differentiation switches can be turned on or the cells are quiescence while proliferation decreases.

(C) The more positive E_{hc} (GSH) becomes the more differentiation switches are turned on until they reach a maximum where nearly all cells are differentiating.

(D) While cells undergo differentiation, proliferation switches are turned down and finally turned off. Cells that are not terminally differentiated could undergo proliferation with an appropriate signal and associated redox environment. We would expect that these partially differentiated cells might shift to a more negative reduction potential, *i.e.*, to the left.

Quiescent cells are considered to be in this state; however, this state is reversible [38, 39]; quiescent cells can return to a more reduced environment and re-enter the cell cycle; terminally differentiated cells cannot.

(E) If E_{hc} (GSH) becomes too positive, then death signals are activated and apoptosis is initiated. This mechanism provides for the orderly removal of cells that have lost their ability to control their redox environment and therefore, are not functioning normally. It should also coincide with signaling pathways to purposely dispose of unneeded cells.

(F) Very high values of E_{hc} (GSH), *i.e.* more positive values, perhaps resulting from severe oxidative stress, leave only necrosis as a path to cell death.

Figure 9. MnSOD in the mitochondrial matrix is at the bifurcation point between 1-electron and 2-electron signaling

The different signaling cascades provide a feedback mechanism for the high-flux metabolic circuit as well as low flux circuits that lead to gene expression, such as with the HIF pathway as presented in Fig. (2).