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Original Contribution

A New Paradigm: Manganese Superoxide Dismutase Influences the Production of H_2O_2 in Cells and Thereby Their Biological State

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Abstract

The principal source of hydrogen peroxide in mitochondria is thought to be from the dismutation of superoxide *via* the enzyme manganese superoxide dismutase (MnSOD). However, the nature of the effect of SOD on the cellular production of H_2O_2 is not widely appreciated. The current paradigm is that the presence of SOD results in a lower level of H_2O_2 because it would prevent the non-enzymatic reactions of superoxide that form H_2O_2. The goal of this work was to: a) demonstrate that SOD can increase the flux of H_2O_2, and b) use kinetic modelling to determine what kinetic and thermodynamic conditions result in SOD increasing the flux of H_2O_2. We examined two biological sources of superoxide production (xanthine oxidase and coenzyme Q semiquinone, $CoQ^{\bullet-}$) that have different thermodynamic and kinetic properties. We found that SOD could change the rate of formation of H_2O_2 in cases where equilibrium-specific reactions form superoxide with an equilibrium constant (K) less than 1. An example is the formation of superoxide in the electron transport chain (ETC) of the mitochondria by the reaction of ubisemiquinone radical with dioxygen. We measured the rate of release of H_2O_2 into culture medium from cells with differing levels of MnSOD. We found that the higher the level of SOD, the greater the rate of accumulation of H_2O_2. Results with kinetic modelling were consistent with this observation; the steady-state level of H_2O_2 increases if $K<1$, for example $CoQ^{\bullet-}+O_2 \rightarrow CoQ+O_2^{\bullet-}$. However, when $K>1$, *e.g.* xanthine oxidase forming $O_2^{\bullet-}$, SOD does not affect the steady state-level of H_2O_2. Thus, the current paradigm that SOD will lower the flux of H_2O_2 does not hold for the ETC. These observations indicate that MnSOD contributes to the flux of H_2O_2 in cells and thereby is involved in establishing the cellular redox environment and thus the biological state of the cell.
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Keywords: Superoxide dismutase; Mitochondria; Coenzyme Q; Hydrogen peroxide; Superoxide; Redox environment; Free radical

Introduction

Superoxide dismutase is an important antioxidant enzyme as it is found in nearly all organisms. In mammals there are at least three forms of SOD: a cytosolic (CuZnSOD), an extracellular (ECSOD), and a mitochondrial form (MnSOD). All SOD enzymes catalyze the dismutation of superoxide, Reactions (2)–(5) Table 1 [1,2]:

$$O_2^{\bullet-} + O_2^{\bullet-} + 2H^+ \xrightarrow{\text{SOD } k \approx 2\times10^9 M^{-1}s^{-1}} H_2O_2 + O_2$$

[3–5]

It has been shown that MnSOD-knockout mice die within 1–18 days after birth, depending on their genetic background [6,7]; thus, MnSOD is an essential enzyme. Changes in SOD levels inside cells can have opposing effects. High over-expression of SOD in *E. coli* has been found to increase sensitivity to paraquat and to hyperoxia [8], as well as ionizing radiation due to apparent increased production of H_2O_2 [9]. Human and mouse cell clones that overexpress human CuZnSOD appear to have higher levels of hydrogen peroxide [10,11]. However, transfection of V79 Chinese hamster cells to overexpress CuZnSOD resulted in a decrease in H_2O_2 in cells [12]. Overexpression of MnSOD in several human cell lines has provided indirect evidence that this increased expression leads to increases in the production of H_2O_2 [13–16]. However, the current paradigm in the research community is that SOD decreases the production of H_2O_2

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Table 1
The reactions in the kinetic model

Reaction number	Reaction	Rate constant $M^{-1}\cdot s^{-1}$ or s^{-1}	Reference/comment
1a	$XO\text{-}FADH^{\bullet}+O_2 \rightarrow XO\text{-}FAD+O_2^{\bullet-}+H^+$	$k_{1a}=7\times10^4$	[30]
−1a	$XO\text{-}FAD+O_2^{\bullet-}+H^+ \rightarrow XO\text{-}FADH^{\bullet}+O_2$	$k_{-1a}=7$	Estimated here using $K_{1a}=10^4$
1b	$CoQ_{10}^{\bullet-}+O_2 \rightarrow CoQ_{10}+O_2^{\bullet-}$	$k_{1b}=8\times10^3$	[74,92–94] The actual value is uncertain, but an equilibrium constant appears to be ≈0.01–0.1.
−1b	$CoQ_{10}+O_2^{\bullet-} \rightarrow CoQ_{10}^{\bullet-}+O_2$	$k_{-1b}=8\times10^5$	[74,92,93]
2	$Mn^{III}SOD+O_2^{\bullet-} \rightarrow Mn^{III}SOD{:}O_2^{\bullet-}$ [a]	$k_2=1.5\times10^9$	[96,97]
−2	$Mn^{III}SOD{:}O_2^{\bullet-} \rightarrow Mn^{III}SOD+O_2^{\bullet-}$	$k_{-2}=3.5\times10^4$	[96]
3	$Mn^{III}SOD{:}O_2^{\bullet-} \rightarrow Mn^{II}SOD+O_2$	$k_3=2.5\times10^4$	[96]
−3	$Mn^{II}SOD+O_2 \rightarrow Mn^{III}SOD{:}O_2^{\bullet-}$ [a]	$k_{-3}=0$	[96]
4	$Mn^{II}SOD+O_2^{\bullet-} \rightarrow Mn^{II}SOD{:}O_2^{\bullet-}$ [b]	$k_4=1.5\times10^9$	[95,96]
−4	$Mn^{II}SOD{:}O_2^{\bullet-} \rightarrow Mn^{II}SOD+O_2^{\bullet-}$	$k_{-4}=3.5\times10^4$	[96]
5	$Mn^{II}SOD{:}O_2^{\bullet-}+2H^+ \rightarrow Mn^{III}SOD+H_2O_2$	$k_5=2.5\times10^4$	[96]
−5	$Mn^{III}SOD+H_2O_2 \rightarrow Mn^{II}SOD{:}O_2^{\bullet-}+2H^+$	$k_{-5}=300$	[96]
6	$Mn^{II}SOD{:}O_2^{\bullet-} \rightarrow DEP$ [c]	$k_6=650$	[96]
−6	$DEP \rightarrow Mn^{II}SOD{:}O_2^{\bullet-}$	$k_{-6}=10$	[96]
7	$2H^++2O_2^{\bullet-} \rightarrow O_2+H_2O_2$	$k_7=2.4\times10^5$	[5]
−7	$O_2+H_2O_2 \rightarrow 2H^++2O_2^{\bullet-}$	$k_{-7}=0$	[5]
8	$GPx_r+H_2O_2+H^+ \rightarrow GPx_0+H_2O$ [d]	$k_8=2.1\times10^7$	[97–99]
−8	$GPx_0+H_2O \rightarrow GPx_r+H_2O_2$	$k_{-8}=0$	[97,98]
9	$GPx_0+GSH \rightarrow GSGPx+H_2O$	$k_9=4\times10^4$	[97,98]
−9	$GSGPx+H_2O \rightarrow GPx_0+GSH$	$k_{-9}=0$	[97,98]
10	$GSGPx+GSH \rightarrow GPx_r+GSSG+H^+$	$k_{10}=1\times10^7$	[97,98]
−10	$GPx_r+GSSG+H^+ \rightarrow GSGPx+GSH$	$k_{-10}=0$	[97,98]
11	$GSSG \rightarrow 2GSH$		
−11	$2GSH \rightarrow GSSG$		
12	$CoQ^{\bullet-}+O_2^{\bullet-}+2H^+ \rightarrow CoQ+H_2O_2$	$k_{12(obs)}=3\times10^6$ [e]	Estimated

[a] $Mn^{III}SOD{:}O_2^{\bullet-}$ is a complex of $Mn^{III}SOD$ and $O_2^{\bullet-}$.
[b] $Mn^{II}SOD{:}O_2^{\bullet-}$ is a complex of $Mn^{II}SOD$ and $O_2^{\bullet-}$.
[c] DEP is dead end product.
[d] Catalase was not included in the kinetic model because it is primarily a peroxisomal enzyme. We included only GPx1 as a sink for H_2O_2. Because we kept the capacity of the system for removing H_2O_2 constant, the inclusion of catalase or peroxiredoxin-III as additional components of the H_2O_2-removing system would make no difference in the final results of the model.
[e] This is an estimate based on the non-enzymatic dismutation of superoxide. The rate constant will rely on the pH and pK_a of these two species. If we assume the maximum rate constant is parallel to that of superoxide, *i.e.* the reaction of protonated and unprotonated species, then we can assume that the fastest reaction will have either $O_2^{\bullet-}$ protonated or $CoQ^{\bullet-}$ protonated. If the effective pK_a of $CoQH^{\bullet}$ is 5.9 [100] then at pH 7.4 about 3% of $CoQ^{\bullet-}$ and 0.2% of $O_2^{\bullet-}$ will be protonated. With this, an estimate of an observed rate constant would be $k_{obs}\approx3\times10^6\ M^{-1}\ s^{-1}$. Once superoxide is formed, possible routes to formation of H_2O_2, as a rate equation, are: $+d[H_2O_2]/dt=\{k_{SOD}\ [SOD]+k_{CoQ^{\bullet-}}\ [CoQ^{\bullet-}]+k_{dismut}[O_2^{\bullet-}]+k_{other}[other]\}\ [O_2^{\bullet-}]$. Estimating the contributions of each term $+d[H_2O_2]/dt=\{2\times10^4\ s^{-1}+3\times10^{-1}\ s^{-1}+2.4\times10^{-4}\ s^{-1}\times+k_{other}[other]\}\times[O_2^{\bullet-}]$, and assuming that $[O_2^{\bullet-}]$ is at most on the order of 1 nM, we see that the termination reaction in question is negligible, as well as the chemical dismutation of superoxide. Thus this reaction was not included in the kinetic model. Here, "other" refers to reactions of superoxide with substances such as aconitase, Fe^{3+}cytochrome *c*, nitric oxide. These represent a small fraction of the reactions of superoxide [101,102].

[17–19]: "the proposal that SOD enhances H_2O_2 by catalyzing the dismutation reaction can be discounted" [17].

The current paradigm

The concentrations of $O_2^{\bullet-}$ and H_2O_2 in a cell are assumed to be in a quasi steady-state. These steady-state concentrations, $[O_2^{\bullet-}]_{ss}$ and $[H_2O_2]_{ss}$, reflect a balance between the rate of formation and the rate of removal. Thus, the steady-state level can change by either changing the rate of formation and/or the rate of removal. It is widely accepted that changes in levels of cellular SOD will result in:

a. a change in $[O_2^{\bullet-}]_{ss}$, *e.g.* an increase in SOD would increase the rate of removal of $O_2^{\bullet-}$ and thereby lower $[O_2^{\bullet-}]_{ss}$;
b. no change in the rate of production of $O_2^{\bullet-}$;
c. a minor change in the rate of production of H_2O_2, no more than a factor of two (This assumes that *Case 3* described below is not applicable.); this change in the rate of production of H_2O_2 could result in
d. a minor change in $[H_2O_2]_{ss}$ [17].

This paradigm is based on the observation that the rate of production of H_2O_2 by the enzyme xanthine oxidase (XO) is not affected by SOD. XO is a well-studied enzyme that is widely used as a tool to generate superoxide and hydrogen peroxide in experimental systems [20].

A new paradigm

However, we propose that there are other superoxide-generating systems (*e.g.* the mitochondrial electron transport

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