MODELING PEROXIDASE-OXIDASE INTERACTIONS

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ABSTRACT

Reactive oxygen species (ROS) and peroxidase-oxidase (PO) reactions are Janus-faced contributors to cellular metabolism. At low concentrations, reactive oxygen species serve as signaling molecules; at high concentrations, as destroyers of proteins, lipids and DNA. Correspondingly, PO reactions are both sources and consumers of ROS. In the present paper, we study a well-tested model of the PO reaction based on horseradish peroxidase chemistry. Our principal predictions are these: 1. Under hypoxia, the PO reaction can emit pulses of hydrogen peroxide at apparently arbitrarily long intervals. 2. For a wide range of input rates, continuing infusions of ROS are transduced into bounded dynamics. 3. The response to ROS input is hysteretic. 4. With sufficient input, regulatory capacity is exceeded and hydrogen peroxide, but not superoxide, accumulates. These results are discussed with regard to the episodic nature of neurodevelopmental and neurodegenerative diseases that may result in positive feedback and pathology of increasing severity.

INTRODUCTION

Reactive Oxygen Species (ROS)

The traditional view [1] of these molecules is that they are harmful by-products of oxidative metabolism. If allowed to accumulate, they damage cells and the biomolecules from which they are constructed. Excessive levels of ROS have also been linked to oxidative stress and various diseases [2].

At low concentrations, reactive oxygen species also function as signaling molecules and secondary messengers that affect multiple metabolic pathways, induce or suppress gene expression and activate a variety of signaling cascades [1-4]. Both views underscore the importance of ROS regulation.

Peroxidase Enzymes

These multifunctional enzymes catalyze a remarkable diversity of reactions. Some promote normal physiological function including defense against pathogens, in which capacity they function as part of the innate immune system [5]. But like reactive oxygen species, peroxidase enzymes can also be a source of dysfunction. Both myeloperoxidase (MPO) and eosinophil peroxidase (EP) are sources of potent oxidants (hydroxyl radical, singlet oxygen, etc.) that destroy pathogens via halogenation and nitration of their proteins [6-8] in the first instance, and humoral immunity, in the second. Not surprisingly, excessive production of these compounds is detrimental to cell function and survival. MPO, in particular, is recognized as a major player in inflammation and has further been linked to various allergic, psychiatric and neurodegenerative diseases [9]. In some cases, these disorders are episodic [10, 11], an observation suggesting the possibility of non-equilibrium dynamics and inherent cyclicity.

In this paper, we study a well-tested peroxidase-oxidase oscillator based on horseradish peroxidase chemistry. Our principal results are as these: 1. For suitably chosen parameter values, pulsatile bursts of hydrogen peroxide are produced. These may relate to episodic symptomatology in diseases such as multiple sclerosis (MS) and Amyotrophic lateral sclerosis (ALS) in which oxidative stress is known to play a role [10, 14].
2. The PO reaction can transduce continuing inputs of hydrogen peroxide and superoxide that would otherwise accumulate, into bounded dynamics for a wide range of input rates. As in any homeostatic system, regulatory capacity can be exceeded. In this case, hydrogen peroxide concentrations can increase without bound. We further suggest that downstream interactions with other metabolic systems can cause positive feedback and pathology of increasing severity.

PEROXIDASE-OXIDASE REACTION

With NADH serving as hydrogen donor, the overall stoichiometry is

\[ 2NADH + O_2 + 2H^+ \rightarrow 2NAD^+ + 2H_2O \quad (R_0) \]

In vitro, Reaction (I) is most often studied with continuing inputs of NADH and oxygen. Under these circumstances, and in the presence of modifiers such as dichlorophenol (DCP), and methylene blue (MB), the concentrations of \( O_2 \), NADH, and various enzyme intermediates can oscillate. In detail, the reaction can manifest a variety of nonlinear behaviors including simple, period-doubled, relaxation and mixed mode oscillations, quasiperiodicity and chaos. Together with bistability, which is important to the results that follow, these behaviors are reproducible by detailed models [15, 16] that incorporate the mechanistic essentials and utilize defensible parameter values.

Mechanism

 Whereas the balance equations of PO reactions are simple, the underlying mechanisms (Table I) are not. Typically, they involve active species such as \( H_2O_2 \), NAD\(^+\), and \( O_2^- \) and multiple enzyme states that differ according to oxidation state: compound I (coI or Per\(^{3+}\)), compound II (coII or Per\(^{4+}\)), ferric peroxidase (Per\(^{3+}\)), ferrous peroxidase (Per\(^{2+}\)), and compound III (coIII or Per\(^{5+}\)) [17]. The reactions by which these species are transformed into each other constitute a pair of oxidation-reduction loops coupled by successive reductions (R\(_3\) and R\(_4\)) of coI and coII. These reactions generate NAD radicals via the oxidation of NADH.

The first of the two feedback loops,

\[ \text{Per}^{3+} \rightarrow \text{col} \rightarrow \text{coII} \rightarrow \text{Per}^{3+} \quad (R_{\text{IIa}}) \]

is the “peroxidase cycle.” Here, \( H_2O_2 \) oxidizes to col, which is then converted back to Per\(^{3+}\). The second feedback loop, the “oxidase cycle,” is actually two pathways:

\[ \text{Per}^{3+} \rightarrow \text{coII} \rightarrow \text{col} \rightarrow \text{Per}^{3+} \quad (R_{\text{IIb}}) \]

and

\[ \text{Per}^{3+} \rightarrow \text{Per}^{2+} \rightarrow \text{coIII} \rightarrow \text{col} \rightarrow \text{Per}^{3+} \quad (R_{\text{IIIb}}) \]

Here, Per\(^{3+}\) is oxidized to coIII, which is then reduced to (R\(_3\)). In the first case (R\(_{\text{IIa}}\)), the transformation is direct; in the second (R\(_{\text{IIIb}}\)), via formation of Per\(^{2+}\).

Summing elementary steps yields the following:

1. Peroxidase cycle (R\(_1\) + R\(_3\) + R\(_4\)): \( 2NADH + O_2 + 2H^+ \rightarrow 2NAD^+ + 2H_2O \) (R\(_0\))

2. Oxidase cycle with Per\(^{2+}\) (R\(_{10}\) + R\(_{11}\) + R\(_8\) + R\(_3\) + R\(_4\)) or without (R\(_8\) + R\(_6\) + R\(_3\) + R\(_4\)) plus R\(_5\):

\[ 2NADH + O_2 + 2H^+ \rightarrow 2NAD^+ + 2H_2O \quad (R_i) \]

In short, NADH is oxidized to NAD\(^+\) by the peroxidase cycle, and to NAD\(^+\) by the oxidase cycle. In the former case, the oxidant is hydrogen peroxide; in the latter, molecular oxygen.

METHODS

Gear integration was used to generate numerical solutions. Forward and backward (parameter increasing or decreasing) “brute force” bifurcation diagrams were computed using transients of 50,000 and 1,000,000 seconds. This allowed us to distinguish asymptotic from transient dynamics. Additionally:

1. For the first parameter value, we used standard laboratory start-up conditions: \([O_2] = 16.7 \mu\text{mol/l}; \text{peroxidase} (0.4 \mu\text{mol/l})\) in its native (Per\(^{3+}\)) state and concentrations of the remaining species set to zero. For subsequent parameter values, calculations were initialized with the final concentrations obtained for the previous value.

2. For each parameter value, 128 post-transient points were recorded in the case of equilibria, and a like number of successive maxima, in the case of oscillatory solutions.

3. To deal with hydrogen peroxide blow-up, we trapped for diverging solutions during the post-transient phase of the calculations. When the trap value was exceeded, the computation was halted, and initial concentrations for the next parameter value reset to the standard start-up conditions. The criterion for blow-up was \([H_2O_2] > 100 \mu\text{mol/l}\), substantially greater than levels observed in the absence of ROS input.

RESULTS

Long-Term Cycling

Figures 1 and 2 illustrate the consequences of reducing \( k_1 \), the rate constant associated with NADH autoxidation. With \( k_1 \) sufficiently small, oscillations with an average period of nearly four days were observed. The presumptive basis of this behavior is near-heteroclinicity. By this we mean that the motion is determined by a pair of saddle foci, and that with decreasing values of \( k_1 \), the asymptotic dynamics approach a heteroclinic orbit (HC in the figure) that connects them. The period on such a connection is infinite, suggesting the possibility of cycles of arbitrarily long period. Importantly, the cycles manifest as bursts of hydrogen peroxide that can induce downstream pathology.
Response to ROS Input

The response to varying hydrogen peroxide input is shown in Figure 3. Here we display forward and backward bifurcation diagrams where the input rate is the bifurcation parameter. The diagrams can be divided into four regions. In Region I, solutions tend to a periodic orbit as indicated by successive maxima in \([\text{H}_2\text{O}_2]\). In Region II, the periodic attractor coexists with a stable point, and there is hysteresis. In Region III, only the point attractor is evident. In Region IV, hydrogen peroxide concentration increases without apparent bound. In this last region, the hydrogen peroxide curve is no longer a sequence of attractors, but one of transients. This is confirmed by the observation that hydrogen peroxide concentration increases with transient length.

Coincident with hydrogen peroxide blow-up is shutdown of the oxidase cycle. This follows from the fact that hydrogen peroxide competes with superoxide and \(\text{NAD}^+\) for \(\text{Per}^{3+}\). As \([\text{H}_2\text{O}_2]\) increases, more and more \(\text{Per}^{3+}\) is converted to \(\text{col}\) and less and less to \(\text{coIII}\). Eventually, essentially all \(\text{Per}^{3+}\) is being converted to \(\text{col}\); the oxidase cycle no longer operates and peroxidase cycling is maximal. Further increases in input exceed the peroxidase cycle’s ability to consume hydrogen peroxide, which then accumulates.

In contrast, superoxide concentrations remain bounded in response to external inputs. Essentially, this is because superoxide dismutates (\(R_i\)); more precisely, because its concentration represents a balance between production (\(R_p\) and external input) and consumption (\(R_c + R_{1d}\)). In this case, the oxidase cycle does not quite shut down: some superoxide reacts with \(\text{Per}^{3+}\) and is converted to \(\text{coIII}\). A further consequence (not shown) of dismutation is that superoxide input, like that of hydrogen peroxide, induces apparently unbounded increases in \([\text{H}_2\text{O}_2]\).

DISCUSSION

Long-Term Cycling

It is well known that heteroclinic and homoclinic orbits can induce oscillations of arbitrarily long period. Still, the usual, and usually correct, presumption is that time and length scales vary inversely. From this point of view, the PO reaction’s predicted ability to generate oscillations with periods on the order of days is noteworthy, particularly because the bursting manifests as pulses of hydrogen peroxide (Figures 1, 2). Not only are the latter themselves potentially damaging, but, as discussed below, they can also trigger downstream reactions that feed back to produce additional ROS.

The reality of episodic symptomatology, i.e., “flare-ups”, in MS and ALS is well-established [10, 14]. Likewise, behavioral and metabolic lability on time scales of days to months is also characteristic of ASD (Pers. Obs.). Such variability may reflect near-heteroclinic or near-homoclinic forcing of the sort discussed here. Alternatively, long-term metabolic and behavioral cycling may be consequent to subharmonic resonance involving either exogenous periodic forcing or interaction with other systems. In the PO reaction, such forcing can result directly from light-dark variation [18] and indirectly from diurnal fluctuation in the concentration of cofactors such as melatonin [19]. Other possible sources of subharmonic resonance are glycolytic [20] and mitochondrial [21] oscillators. In passing, we note that peroxidase loses activity in vitro on time scales 6-12 h; that hydrogen peroxide promotes enzyme inactivation via reaction with \(\text{col}\) and that inactivated peroxidase can itself promote neuroinflammation [9, 22, 23].

ROS Regulation

According to our model, the PO reaction transduces unbounded accumulation of hydrogen peroxide and superoxide into bounded dynamics for a wide range of input rates. This is shown for representative time series (log input rate = -3.25 \(\mu\)M/s) in Figures 3c and 4c. Here we compare ROS concentrations that would obtain absent the PO reaction with those observed in its presence. Because ROS are produced both enzymatically and non-enzymatically, we compare accumulated inputs with concentrations observed for various subsets of the full mechanism. In the case of hydrogen peroxide, there are two principal sources of input: external and reaction \(R_1\) (autoxidation of NADH). Absent enzymatic reactions (“No Enzyme”; “No Enzyme, No Input” in Figure 3c), total \(\text{H}_2\text{O}_2\) accumulations consequently exceed the accumulated external inputs. In the case of superoxide input, non-enzymatic production requires presence of the enzyme, i.e., molecular oxygen reacts with NAD radicals to produce superoxide (\(R_3\)), but the production of NAD radicals requires reaction of NADH with \(\text{col}\) and \(\text{coII}\) (\(R_3\) and \(R_4\)). Hence the accumulated external input equals superoxide concentration sans enzyme. With enzymatic reactions added back in (“full system”), both \([\text{H}_2\text{O}_2]\) and \([\text{O}_2^-]\) are substantially less than the accumulated inputs.

Extended System

Now consider peroxidase up-regulation by \(\text{H}_2\text{O}_2\) [24]. In response to increasing \(\text{H}_2\text{O}_2\) input, and provided that enzyme concentration changes slowly, the system may switch from Region I to Regions II or III. The increase in \([\text{H}_2\text{O}_2]\) is reversible, but will be delayed due to hysteresis. Elsewhere, we document that mean hydrogen peroxide concentrations are higher under fixed point dynamics when these coexist with cycles. Then, increasing ROS input can induce prolonged departures from baseline conditions before the latter are restored. When extended to include enzyme up-regulation, the system thus functions as an excitable medium.

Neurologic Disease

Reduced concentrations of oxidant scavengers and inflammatory response have been associated with a variety of neurodegenerative and neurodevelopmental disorders. The former include Alzheimer’s disease (AD), MS, Huntington’s disease, Parkinson’s disease (PD), ALS [22, 25-28]; the latter,
autism (AD) and related disorders (PDD-NOS) [29-33]. With regard to autism, the fact that PO reactions connect to multiple metabolic pathways involving inflammation, energy production and ammonia detoxification may explain the bewildering array of associated metabolic, neurologic, cognitive and behavioral abnormalities [34, 35]. These comorbidities, it has been suggested [33, 36, 37], may reflect mitochondrial dysfunction, which, in turn, implies elevated concentrations of ROS. In this regard, we note the following:

1. Up-regulation of MPO and inflammatory episodes are well-documented in MS, AD and PD [38-40]. In autism, the evidence is less compelling, but elevated numbers of circulating monocytes and 3-nitrotyrosine (biomarker of inflammation and MPO) have been reported [41, 42].

2. Mild to moderate hyperammonemia (HA) and other correlates of mitochondrial dysfunction have also been observed in ASD [34]. Common manifestations of HA include abnormal sleep patterns, nausea, digestive dysfunction, cognitive and perceptual dysfunction [43-45] and neurological deficits – all frequently reported correlates.

3. Concentrations of scavenging enzymes are substantially lower in brain than in other organs [46], a feature that may facilitate signaling. The disadvantage is increased vulnerability to oxidative stress. Likewise, because they lack a complete urea cycle, brain cells are especially sensitive to HA [45, 47, 48].

Importantly, there are multiple instances of positive feedback involving these and other pathways. For instance:

1. By increasing the fraction of Per$^{3+}$ directly recycled to col, higher concentrations of hydrogen peroxide enhance the production of HOCl, the principal oxidant in MPO-induced chlorination. The result is more cellular and mitochondrial damage, more ROS, and so forth.

2. In the brain, HOCl, increases ROS concentrations by inactivating glutathione peroxidase and reducing concentrations of glutathione [26]. Again, the result is more ROS, more damage, etc.

3. Electron transport chain (ETC) inhibition results in increased H$_2$O$_2$, which inhibits $\alpha$-ketoglutarate dehydrogenase ($\alpha$-KGDH) activity. This leads to the production of additional H$_2$O$_2$ [50] and reduced TCA cycling [51]. It also leads to increased MPO because the latter is up-regulated by hydrogen peroxide [24]. Increased H$_2$O$_2$ further exacerbates complex I deficiency, which, via the steps enumerated, leads to more MPO, more chlorination products and more inflammation.

4. ETC inhibition also reduces NADH/NAD$^+$ ratios, further blocking the TCA cycle. Since ammonia detoxification in brain utilizes TCA cycle reactions [47, 48], reduced TCA cycling leads to HA, which, in turn, promotes the production of O$_2$ and H$_2$O by inhibition of $\alpha$-KGDH [52].

5. HOCl also inhibits glycolytic output via inactivation of glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase [25]. This further impedes TCA cycling, ammonia detoxification, etc.

These observations suggest that the argument for mitochondrial dysfunction can be stood on its head. Oxidative stress can as easily be a cause as a consequence, i.e., ROS impairs mitochondrial function; mitochondrial dysfunction produces ROS. In both instances, one expects the same suite of correlated abnormalities. In sum, positive feedback can produce similar symptomatology for multiple primary defects.

REFERENCES


**TABLE 1. ELEMENTARY STEPS.**

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Constant</th>
</tr>
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<tbody>
<tr>
<td>1. NADH + O₂ + H⁺ → NAD⁺H₂O₂</td>
<td>3.0 M⁻¹s⁻¹</td>
</tr>
<tr>
<td>2. H₂O₂ + Per³⁻ → col</td>
<td>1.8×10⁴ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>3. col + NADH → colII + NAD⁺</td>
<td>4.0×10⁴ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>4. colII + NAD⁺ → Per³⁻+NAD⁺</td>
<td>2.6×10⁴ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>5. NAD⁺+ O₂ → NAD⁺+O₂⁻</td>
<td>2.0×10⁵ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>6. O₂⁻ + Per³⁻ → colIII</td>
<td>1.7×10⁵ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>7. 2O₂⁻ + 2H⁺ → H₂O₂+O₂</td>
<td>2.0×10⁵ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>8. colIII + NAD⁺ → col + NAD⁺</td>
<td>1.75×10⁸ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>9. 2NAD⁺ → (NAD)₂</td>
<td>1×10⁸ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>10. Per³⁻+ NAD⁺ → Per³⁻+NAD⁺</td>
<td>1.8×10⁸ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>11. Per⁺ + O₂ → colIII</td>
<td>1.0×10⁸ M⁻¹s⁻¹</td>
</tr>
<tr>
<td>12. NADH (stock) → NAD⁺</td>
<td>1.143×10⁷ Ms⁻¹</td>
</tr>
<tr>
<td>13. O₂ (gas) → O₂ (liquid)</td>
<td>6.24×10⁸ Ms⁻¹</td>
</tr>
<tr>
<td>14. NADH + H⁺ + O₂⁻ → NAD⁺ + H₂O₂</td>
<td>3.0×10⁸ M⁻¹s⁻¹</td>
</tr>
</tbody>
</table>
FIGURE 1. EFFECT OF REDUCING NADH OXIDATION. a. \( k_1 = 3 \text{ M}^{-1}\text{s}^{-1} \); b. \( k_1 = 3 \times 10^{-2} \text{ M}^{-1}\text{s}^{-1} \); c. \( k_1 = 3 \times 10^{-8} \text{ M}^{-1}\text{s}^{-1} \). \([E_T] = 0.4 \mu\text{M/L}.

FIGURE 2. NADH OXIDATION. (CONTINUED). a. \( k_1 = 3 \times 10^{-2} \text{ M}^{-1}\text{s}^{-1} \) (MAGNIFIED). b. PHASE SPACE PROJECTION, \( k_1 = 3 \times 10^{-1} \text{ M}^{-1}\text{s}^{-1} \); c. MAGNIFICATION OF b. \([E_T] = 0.4 \mu\text{M/L}. \) ONE OF TWO NEAR-HETEROCLINIC ORBITS INDICATED BY “HC.”
FIGURE 3. RESPONSE TO CONTINUING HYDROGEN PEROXIDE INPUT. a, b. BIFURCATION DIAGRAMS. c. TIME SERIES COMPARING ACTUAL CONCENTRATIONS WITH ACCUMULATED INPUTS.

FIGURE 4. RESPONSE TO CONTINUING SUPEROXIDE INPUT. a, b. BIFURCATION DIAGRAMS. c. TIME SERIES COMPARING ACTUAL CONCENTRATIONS WITH ACCUMULATED INPUTS.