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ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY · JUNE 1995

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### Period-Doubling Bifurcations and Chaos in a Detailed Model of the Peroxidase–Oxidase Reaction

T. V. Bronnikova,<sup>†,‡</sup> V. R. Fed'kina,<sup>†,‡</sup> W. M. Schaffer,<sup>†,§</sup> and L. F. Olsen<sup>\*,†</sup>

*Physical Biochemistry Group, Institute of Biochemistry, Odense University, Forskerparken 10, DK-5230 Odense M, Denmark; Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow Region, 142292, Russia; and Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721*

*Received: February 27, 1995; In Final Form: April 13, 1995*<sup>®</sup>

We propose a detailed model of the peroxidase–oxidase reaction, which is capable of reproducing many of the nonlinear behaviors observed in the experimental system. These include simple and complex periodic oscillations, period doubling bifurcations, chaotic dynamics, and transient chaos.

#### Introduction

The peroxidase–oxidase (PO) reaction is the peroxidase-catalyzed oxidation of reduced nicotinamide adenine dinucleotide (NADH) by molecular oxygen:



When NADH and O<sub>2</sub> are continuously supplied to a reaction mixture containing the enzyme, the system may exhibit damped and sustained oscillations.<sup>1,2</sup> Furthermore, in the presence of the modifiers 2,4-dichlorophenol and methylene blue, both complex periodic and chaotic oscillations may be observed.<sup>3,4</sup> Several mechanistic models have been proposed to explain the experimental observations of complex dynamics.<sup>5–11</sup> These models can be divided into two classes: (i) simple abstract models which contain only the essential features of the reaction but ignore most of the individual steps;<sup>5,6</sup> (ii) detailed models which attempt to incorporate all the important elementary steps

into the reaction scheme.<sup>7–11</sup> Although both types of models are capable of exhibiting complex dynamics, including chaos, the simple models have, up to now, outperformed the detailed models in reproducing the reaction's individual features, such as the waveform of the oscillations and their nonuniformity.<sup>12</sup> Furthermore, in the detailed models most of the rate constants used to obtain complex dynamics are not in accordance with the experimentally determined rate constants. Here we present a detailed model of the PO reaction which is capable of simulating the recent period doubling experiments of Geest et al.,<sup>13</sup> using the proper rate constants and the same conditions (enzyme concentration, O<sub>2</sub> and NADH supply rates) as in the experiments. The model is a slight extension of a model recently proposed by Olson et al.<sup>11</sup> under the name Urbanalator.

#### The Model

The model is built using the same elementary reaction steps as in previous detailed models such as the Yokota–Yamazaki (YY) scheme,<sup>7</sup> the Fedkina–Ataullakhanov–Bronnikova (FAB) scheme,<sup>8</sup> the so-called Models A and C,<sup>9,10</sup> and the recent Urbanalator model.<sup>11</sup> The model is listed below:

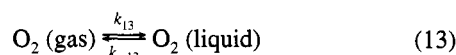
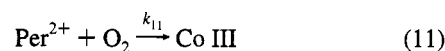
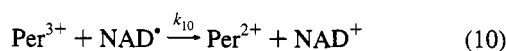
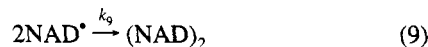
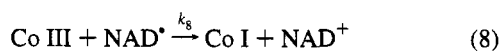
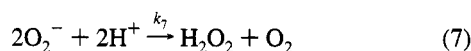
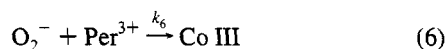
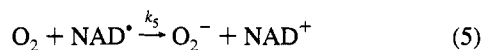
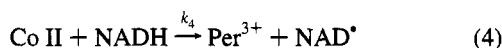
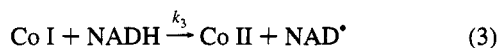
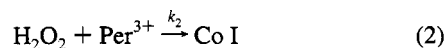


<sup>†</sup> Odense University.

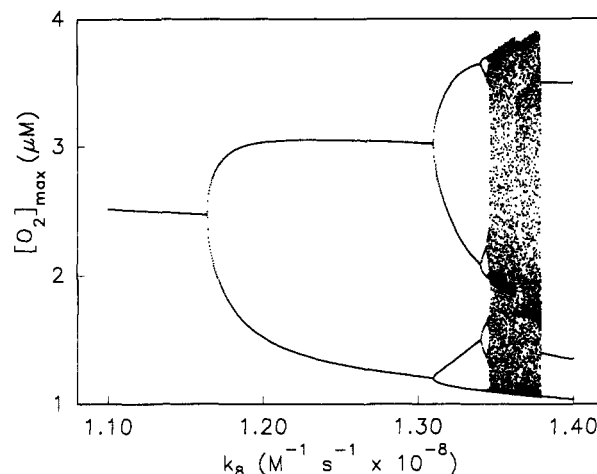
<sup>‡</sup> Russian Academy of Sciences, Pushchino.

<sup>§</sup> University of Arizona.

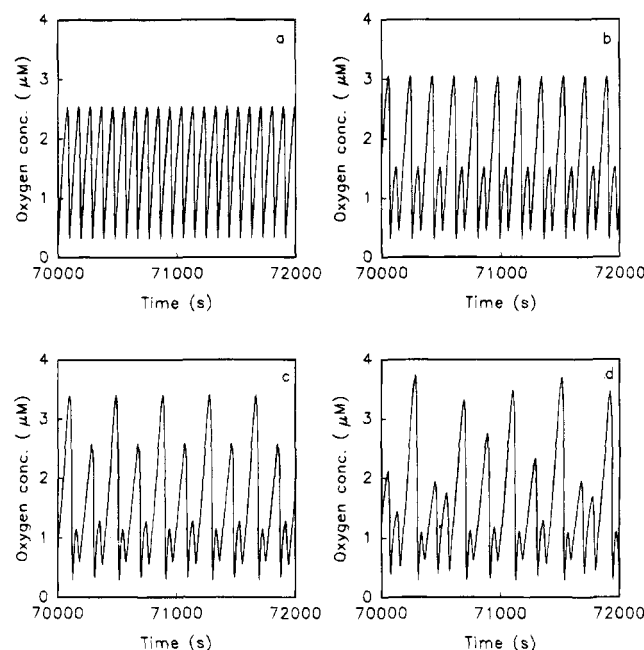
<sup>®</sup> Abstract published in *Advance ACS Abstracts*, June 1, 1995.



Here  $\text{Per}^{3+}$  and  $\text{Per}^{2+}$  indicate ferric peroxidase and ferrous peroxidase respectively. Co I, Co II, and Co III indicate the enzyme intermediates compound I, compound II, and compound III. Reaction 1 is a nonenzymatic oxidation of NADH by  $\text{O}_2$ . It is known to be catalyzed by methylene blue<sup>14,15</sup> which is also present in the experiments.<sup>3,11–13</sup> It first appeared in the Urbanalator model.<sup>11</sup> Reactions 2–4 comprise the classical peroxidase catalytic cycle. Reactions 5–7 and reaction 9 are well-documented processes involving free radicals.<sup>11,16</sup> Reaction 8 was originally proposed by Yokota and Yamazaki,<sup>7</sup> but its rate constant has never been determined directly. Reaction 10 is usually believed to be very slow, since only trace amounts of ferrous peroxidase have been observed in the PO reaction catalyzed by horseradish peroxidase.<sup>7,11</sup> However, recently significant amounts of ferrous peroxidase were measured in an oscillating PO reaction catalyzed by peroxidase from *Coprinus cinereus* (U. Kummer, K. R. Valeur, G. Baier, K. Wegmann, and L. F. Olsen, manuscript in preparation). Furthermore, pulse radiolysis experiments have demonstrated a reduction of ferric peroxidase by  $\text{NAD}^\bullet$ .<sup>17</sup> Reactions 12 and 13 represent the continuous supplies of NADH and oxygen, respectively: NADH is supplied by pumping a concentrated stock solution into the reaction mixture through a capillary at a constant rate, whereas  $\text{O}_2$  is entering the reaction mixture from the gas phase above the liquid, whose composition is controlled by mixing  $\text{O}_2$  and  $\text{N}_2$  using a gas mixer.<sup>3,11–13</sup> Hence the model is not radically different from previous detailed models. It differs from the Urbanalator model<sup>11</sup> by the inclusion of reactions 10 and 11. This parallels the construction of an earlier model of the PO reaction with chaotic dynamics<sup>10</sup> from a model showing only limit cycle oscillations.<sup>9</sup> However, like the Urbanalator model, the present model *does not* include the oxidation of NADH by superoxide, which plays a prominent role in most other detailed models.<sup>7–10</sup> The modifiers methylene blue (MB) and 2,4-dichlorophenol (DCP) are not incorporated directly into the model. However, as mentioned above the value of  $k_1$  is proportional to the concentration of MB. The role of DCP is more difficult to assess. There are no experimental observations which demonstrate that DCP undergoes oxidation–reduction reactions in the PO reaction. However, Halliwell<sup>18</sup> as well as others<sup>2,3</sup> have shown that DCP stimulates the degradation of compound III. In the absence of any other evidence we shall



**Figure 1.** Bifurcation diagram of the detailed peroxidase–oxidase model. Maxima of oscillations of  $\text{O}_2$  are plotted against the corresponding value of  $k_8$ . Other parameters as in “Choice of Rate Constants”.

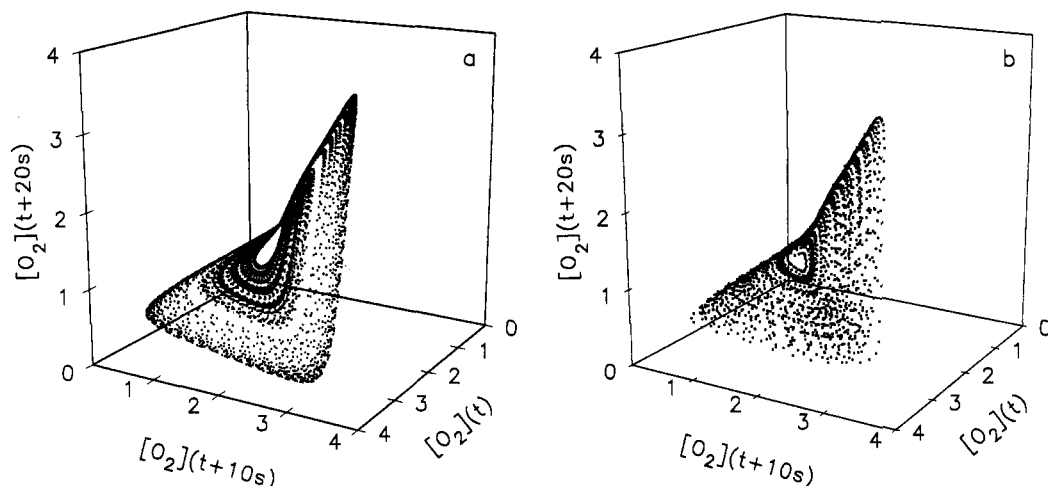


**Figure 2.** Time series from the detailed peroxidase–oxidase model. Oscillations in oxygen concentration are shown for four different values of  $k_8$ : (a)  $1.1 \times 10^8$ , (b)  $1.2 \times 10^8$ , (c)  $1.317 \times 10^8$ , and (d)  $1.35 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ .

assume that the role of DCP in the PO reaction is to increase the value of  $k_8$ , either in a direct chemical way or by inducing a conformational change of the enzyme.

#### Choice of Rate Constants and Numerical Simulations.

Except for  $k_8$ , most of the rate constants of the model have been determined experimentally. Olson et al.<sup>11</sup> proposed a value for  $k_1$  of  $33 \text{ M}^{-1} \text{ s}^{-1}$  at pH 5.1. However, this value is an extrapolation of a rate constant measured by Sevcik and Dunford<sup>15</sup> at pH 9.0 assuming first-order dependence on the concentration of protons. If this is not the case a somewhat smaller value for  $k_1$  can be expected at pH 5.1. The rate constants  $k_2$ – $k_4$  and  $k_6$ – $k_9$  used here are the same as those used for the Urbanalator model.<sup>11</sup> Land and Swallow<sup>19</sup> have estimated a value for  $k_5$  of  $2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  at pH 8.6. Here we assume that  $k_5$  is considerably smaller at pH 5.1. To obtain periodic oscillations, the value of  $k_5$  is not critical. However, to simulate complex dynamics a value for  $k_5$  of the order of  $10^7$ – $10^8$  must be assumed. As opposed to Yokota and

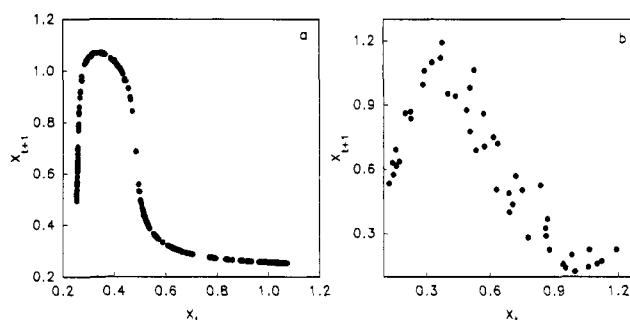


**Figure 3.** Three-dimensional phase plots of the reconstructed time series of  $O_2$  for (a) data from the detailed model with  $k_8 = 1.35 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  and (b) experimental data. The delay used for the reconstruction was 10 s. Concentrations are in  $\mu\text{M}$ . The experiment was performed in a 7 mL sample containing 0.7  $\mu\text{M}$  horseradish peroxidase, 0.1  $\mu\text{M}$  methylene blue, 36  $\mu\text{M}$  dichlorophenol, 0.1 M sodium acetate, pH 5.1. Oxygen entered the reaction mixture from a gas head space above the surface, containing 1.42%  $O_2$  (v/v). The oxygen transfer constant was  $3.7 \times 10^{-3} \text{ s}^{-1}$ . NADH was supplied as a concentrated solution of 0.2 M at constant rate of 18  $\mu\text{L/h}$ , corresponding to an NADH influx of  $1.43 \times 10^{-7} \text{ M/s}$ , i.e., 25% higher than that used in the numerical simulations and in the experiments of Geest et al.<sup>13</sup>

Yamazaki,<sup>7</sup> who concluded that  $k_{10}$  was relatively low, Kobayashi et al.<sup>17</sup> have determined  $k_{10}$  by pulse radiolysis as  $8 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$  at pH 7.0. We believe that the value of  $k_{10}$  is much lower at pH 5.1. Otherwise ferrous peroxidase would be the dominating enzyme intermediate during the reaction, which is not consistent with experimental observations.<sup>2,3,7,11</sup> Therefore we have chosen a value for  $k_{10}$  which is somewhat in between the estimates by Yokota and Yamazaki<sup>7</sup> and Kobayashi et al.<sup>16</sup>  $k_{11}$  was chosen roughly identical to the value reported by Phelps et al.<sup>20</sup> The constants  $k_{11}$  and  $k_{12}$  were chosen so they matched the experimental conditions employed by Geest et al.<sup>13</sup> The following second-order rate constants were used:  $k_1 = 3$ ,  $k_2 = 1.8 \times 10^7$ ,  $k_3 = 4 \times 10^4$ ,  $k_4 = 2.6 \times 10^4$ ,  $k_5 = 2 \times 10^7$ ,  $k_6 = 1.7 \times 10^7$ ,  $k_7 = 2 \times 10^7$ ,  $k_8 = 1.1 \times 10^8$ – $1.4 \times 10^8$ ,  $k_9 = 5.6 \times 10^7$ ,  $k_{10} = 1.8 \times 10^6$ , and  $k_{11} = 10^5$ , all in  $\text{M}^{-1} \text{ s}^{-1}$ . The rate of reaction 12 ( $k_{12}[\text{NADH}]_{\text{stock}}$ ) was  $1.14314 \times 10^{-7} \text{ M/s}$ , corresponding to an influx of a stock solution containing 0.08 M NADH into a volume of 7 mL at a rate of 36  $\mu\text{L/h}$ . The first order rate constant  $k_{-13}$  was equal to  $3.73 \times 10^{-3} \text{ s}^{-1}$  and the rate of diffusion of oxygen into the reaction mixture ( $k_{13}[\text{O}_2]_{\text{gas}}$ ) was  $6.2415 \times 10^{-8} \text{ M/s}$  corresponding to an oxygen concentration in the gas phase of 1.46% (v/v). The enzyme concentration was 0.9  $\mu\text{M}$ , which is slightly higher than in the experiments of Geest et al.<sup>13</sup> (0.7  $\mu\text{M}$ ). The numerical simulations were performed using the LSODE implementation of Gear's algorithm.<sup>21</sup> For convenience the second order rate constants listed above were converted to  $\mu\text{M}^{-1} \text{ s}^{-1}$ . Hence the output of the simulation procedure will be concentrations in  $\mu\text{M}$  vs time in seconds. Otherwise parameters were neither dedimensionalized nor scaled in any other way. For all calculations, except for the bifurcation diagrams, the following initial conditions were used:  $[\text{O}_2]_0 = 16.7 \mu\text{M}$ ;  $[\text{Per}^{3+}]_0 = 0.9 \mu\text{M}$ . All other initial concentrations ( $[\text{O}_2^-]_0$ ,  $[\text{H}_2\text{O}_2]_0$ ,  $[\text{NADH}]_0$ ,  $[\text{NAD}^+]_0$ ,  $[\text{Co I}]_0$ ,  $[\text{Co II}]_0$ ,  $[\text{Co III}]_0$ , and  $[\text{Per}^{2+}]_0$ ) are zero. For the bifurcation diagram the final concentrations at one parameter value were used as initial concentrations for the next parameter value. All numerical solutions shown are asymptotic solutions, following a transient period which was eliminated. This period corresponded to 50 000–200 000 s.

## Results and Discussion

Figure 1 shows a bifurcation diagram constructed by plotting the amplitudes of oscillations of oxygen against changing values



**Figure 4.** Return maps constructed from Poincaré sections of the flows in Figure 3. The units of  $X_i$  and  $X_{i+1}$  are dimensionless.

of  $k_8$ . We observe a transition from a periodic oscillation with a single amplitude through period doubling bifurcations to chaos as  $k_8$  is increased. At higher values of  $k_8$  a stable period-3 cycle is observed. Figure 2 shows the oscillations of oxygen at four different values of  $k_8$ . We note that the waveform of the oscillations and the amplitudes are similar to experimental measurements of Geest et al.,<sup>13</sup> which were made using the same conditions as in the model. Figure 3a shows a phase plot of the chaotic oscillations of oxygen from the model constructed by Takens' method of delays<sup>22</sup> (delay = 10 s) and Figure 3b shows the same plot for an experimental time series made under similar conditions. Figure 4 shows return maps of the simulated and experimental chaotic data from Figure 3. We note that both phase plots and return maps are very similar for the simulated and experimental data. Thus, our model is capable of reproducing many of the nonlinear behaviors observed in the experimental system such as the transition to chaos through period doubling bifurcations followed by a stable period-3 cycle.<sup>23</sup> Other features reproduced by this model include transient chaotic oscillations<sup>23</sup> and oscillation steady-state (OS) bistability<sup>24</sup> (data not shown).

However, there are also features of the experimental system which the model apparently cannot reproduce: The model predicts that at the minima of oxygen oscillations up to 15% of the enzyme should be converted to ferrous peroxidase. Such amounts of ferrous peroxidase have not yet been observed with horseradish peroxidase. However, with peroxidase from *Coprinus cinereus* more than 50% of the enzyme is converted into ferrous peroxidase at the minima of  $O_2$  oscillations (U. Kummer,

K. R. Valeur, G. Baier, K. Wegmann, and L. F. Olsen, manuscript in preparation). A more detailed analysis of the spectral changes during oscillations could reveal exactly how much ferrous peroxidase is formed in the reaction catalyzed by horseradish peroxidase. Furthermore, in the experimental system the dynamics change from chaos and complex oscillations to simple periodic oscillations as the enzyme concentration is increased.<sup>3-5</sup> A similar change is not observed with the model. This suggests that at least one more reaction involving the enzyme needs to be incorporated into the model. However, the identification of such a reaction warrants further experimental work.

**Acknowledgment.** This research was supported by the Danish Natural Science Research Council.

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JP9505486