

Perturbations of Simple Oscillations and Complex Dynamics in the Peroxidase–Oxidase Reaction Using Magnetic Fields

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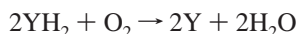
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The effect of dc magnetic fields of the order of 0–4000 G on the oscillating peroxidase–oxidase (PO) reaction was studied. Specifically the effects of magnetic fields on the amplitude of simple periodic oscillations and on complex dynamic states were investigated. Magnetic fields induce a biphasic change in oscillation amplitude: At field strengths up to 1500 G, the amplitude decreases gradually by 11%. At higher field strengths the decrease in amplitude is less, and it vanishes at field strengths of 4000 G and higher. The effect of magnetic fields on complex dynamics is generally to shift the dynamics from one state to a neighboring state. Numerical simulations of a detailed model of the PO reaction support the hypothesis that the magnetosensitive step is the reduction of compound II to ferric peroxidase or the reduction of compound I to compound II or both.

I. Introduction

The peroxidase–oxidase (PO) reaction is the oxidation of an electron donor YH_2 by molecular oxygen, catalyzed by peroxidases.¹



The number of electron donors is limited to a few structurally unrelated organic compounds, one of which is NADH. Many peroxidases from both plant and animals catalyze the PO reaction with NADH as electron donor.^{2,3} In plants this reaction occurs using NADH derived from the oxidation of malate.⁴ When the PO reaction takes place in an open system and in the presence of catalytic amounts of certain phenolic compounds and methylene blue, the concentrations of the reactants NADH and O_2 as well as several enzyme intermediates oscillate.⁵ Both simple periodic oscillations and complex dynamics have been observed,^{5–8} depending on the experimental conditions. Furthermore, such dynamics may be observed over a range of pH values from 5.1 to 6.3.⁹ The dynamics of the PO reaction are very sensitive to even small changes in experimental conditions such as the rates of supply of both NADH and O_2 . In addition, numerical simulations have indicated that the reaction should also be sensitive to small changes in rate constants.^{9,10}

The PO reaction involves some 10–20 elementary reaction steps, and in several of these reactions free radicals are either generated or consumed.¹ In two of the reactions, experimental evidence for a radical pair mechanism has been presented.¹¹ Reactions involving a radical pair mechanism are often sensitive to magnetic fields.^{12,13} The effect of magnetic fields is believed to be due to a change in the rate of recombination of the radical pair. This rate depends on the rate of interconversion of the singlet and triplet spin states of the radical pair. Hence, the application of a magnetic field may change the recombination rate of the radical pair and hence also the rate of the forward

reaction. Given that the dynamics of the PO reaction are very sensitive to changes in rate constants, it is tempting to suggest that the dynamics should be sensitive to magnetic fields. Recently, we observed an effect of static magnetic fields on both simple oscillations and on complex dynamic states of the PO reaction in an open system, where both substrates are supplied continuously to the reaction mixture.¹⁴ Here we wish to report further experimental evidence for the effects of magnetic fields, and in addition we offer a molecular mechanism to explain these effects.

II. Experimental Section

Experiments were performed in a quartz cuvette ($20 \times 20 \times 43.5$ mm) equipped with an oxygen electrode (Microelectrodes Inc., Bedford, NH). The cuvette was placed between the iron poles of a 4 in. horizontal electromagnet (Walker Scientific Inc., Worcester, MA). The residual magnetic flux of the electromagnet, which is considered as zero-field, is 30 G. The static magnetic flux was measured in the middle of the cuvette with a Hall probe connected to a Gaussmeter (F. W. Bell, Orlando, FL). The cuvette is connected through optical fibers to a Zeiss Specord S10 diode array spectrophotometer. Only the photometer's tungsten lamp was used as a light source in order to avoid photochemical reactions induced by UV irradiation.^{15,16} Oxygen concentration, as well as the absorbencies of NADH (at 370 nm), native ferric peroxidase (403 nm), oxyferrous peroxidase (compound III; 418 nm), and ferrous peroxidase (439 nm), was recorded every 2 s and stored in a computer for later analysis.

The reactor had a liquid volume of 8 mL as well as an approximately 9 mL gas phase above the reaction mixture. The reaction mixture contained 0.1 μM methylene blue, 200–600 μM 4-hydroxybenzoic acid or 30–90 μM 4-chlorophenol, and 2.3 μM horseradish peroxidase, in 0.1 M sodium phosphate buffer, pH 6.3. The lower concentrations of 4-hydroxybenzoic acid and 4-chlorophenol were used whenever the desired dynamic state was a simple periodic oscillation. A 0.1 M aqueous solution of NADH was delivered to the reaction vessel

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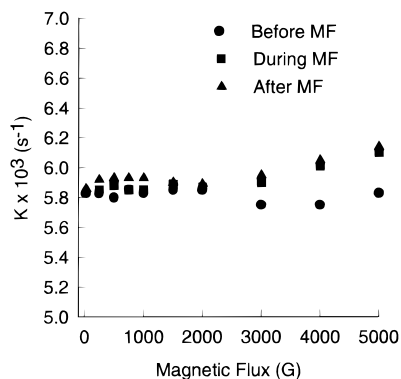


Figure 1. Effect of magnetic fields on the oxygen-transfer constant K . K was measured before (●), during (■), and after (▲) the application of magnetic fields with different magnetic fluxes. The measurements were made in 0.1 M sodium phosphate buffer, pH 6.3.

at constant flow rates through a capillary tube connected to a syringe pump. The volume of the added solution during each experiment was negligible and balanced by an equal volume of water evaporated. Thus, the liquid volume was effectively constant at 8 mL throughout the experiment. Oxygen was supplied at atmospheric pressure to the reaction mixture via a gas mixer. The moisturized O_2/N_2 stream contained 1.05% (v/v) oxygen. The rate of oxygen diffusion ν_{O_2} into the liquid is given by

$$\nu_{O_2} = K([O_2]_{eq} - [O_2])$$

where $[O_2]$ is the oxygen concentration in solution, $[O_2]_{eq}$ is the oxygen concentration at equilibrium, and K is the oxygen-transfer constant. For a stirring rate of 1150 rpm, K was determined to be $5.8 \times 10^{-3} \pm 0.2 \times 10^{-3} s^{-1}$. To investigate the effect of a magnetic field on the oxygen-transfer constant, we measured K before, during, and after the application of magnetic fields from 0 to 5000 G. The result is shown in Figure 1. At field strengths up to 3000 G, there are no significant differences between K values measured in the presence and in the absence of a magnetic field. However, at field strengths above 3000 G, there is a small but significant increase in K after switching on the magnetic field.

Prior to the start of each experiment, the reaction mixture containing enzyme, methylene blue, and phenolic compound (4-hydroxybenzoic acid or 4-chlorophenol) in buffer was thermostated at $28.0 \pm 0.1^\circ C$ and equilibrated with pure nitrogen. Experiments were typically started by adding NADH at a flow rate of $50 \mu L h^{-1}$. As the absorbance at 370 nm reached a level close to the NADH concentration associated with a given dynamic state, the composition of the gas stream was switched from pure N_2 to the O_2/N_2 mixture. Then the NADH flow rate was adjusted ($35\text{--}50 \mu L h^{-1}$) such that the NADH concentration oscillated around a constant mean level corresponding to this particular dynamic state. The dynamics were then recorded over a period of time. Thereafter, the pumping rate was changed to allow the NADH concentration to settle on another mean level associated with a different kind of behavior. Thus, changing the mean NADH concentration allows for the observation of different dynamical states as well as an unambiguous determination of the order in which they occur. Accordingly, we use the mean NADH concentration as a bifurcation parameter. One could also use the NADH flow rate which, of course, determines the average NADH concentration, even though the relation between the two is nonlinear. However, this approach, while it makes for a somewhat more straightforward comparison of the

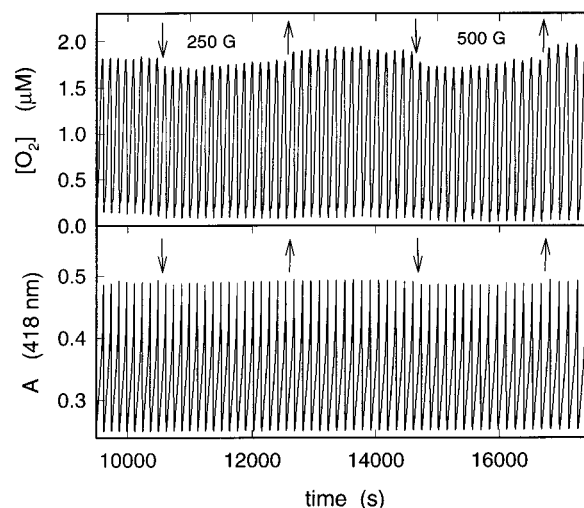


Figure 2. Effect of magnetic fields with field strengths of 250 and 500 G, respectively, on periodic oscillations of O_2 and compound III in the PO reaction. Compound III is measured as the absorbance at 418 nm. The experiment is made in the presence of $200 \mu M$ 4-hydroxybenzoic acid, which ensures that only periodic oscillations are obtained. The inflow rate of NADH was $45 \mu L/h$ corresponding to an average NADH concentration of $100 \mu M$. At the time marked ↓ the magnetic field was turned on, and at the time marked ↑ the field was turned off. Other experimental conditions are provided in the Experimental Section.

data and the simulations, is less reliable. This is because, in spite of great precautions to prepare identical NADH stock solutions, small variations in NADH concentrations are unavoidable. If uncontrolled, these variations will necessarily affect the dynamics and are consequently a potential source of error. In short, the use of mean NADH concentration as described here removes an otherwise inevitable source of interexperiment variability.

Magnetic fields were always switched on and off when the oxygen concentration reached its minimum after a large-amplitude oscillation. The residual magnetic field of the electromagnet was measured as 30 G immediately before switching on the current to the electromagnet. After switching off the current, the magnetic field returned to a value between 25 and 35 G.

Horseradish peroxidase (RZ 3.0) and NADH disodium salt were purchased from Boehringer Mannheim; methylene blue was purchased from Merck. 4-Hydroxybenzoic acid and 4-chlorophenol were kindly provided by the Institute of Chemistry, Odense University.

III. Experimental Results

Sustained oscillations in the PO reaction require the presence of methylene blue and a phenolic compound. Several phenolic compounds and aromatic amines will induce oscillations and complex dynamics.^{17,18} The type of behavior observed depends on the concentration of the phenol¹⁹ in such a way that the higher the concentration of the phenol, the more complex the dynamics. In the study of magnetic field effects on the PO reaction, we begin by examining the effect of magnetic fields on the simple periodic oscillations. Figure 2 shows time series of O_2 and compound III (oxyferrous peroxidase) in the presence of $200 \mu M$ 4-hydroxybenzoic acid. The concentration of 4-hydroxybenzoic acid was adjusted such that only periodic oscillations occur. At the times indicated, magnetic fields of field strengths 250 and 500 G were switched on and off, respectively. We note that the fields induce rapid decreases in amplitudes of O_2

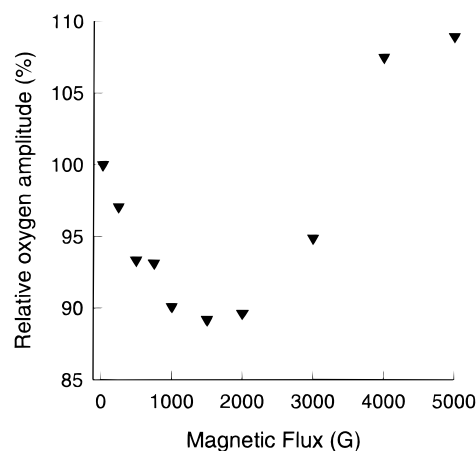


Figure 3. Effect of the magnetic flux density on the O_2 -oscillation amplitude. Experimental conditions as in Figure 2.

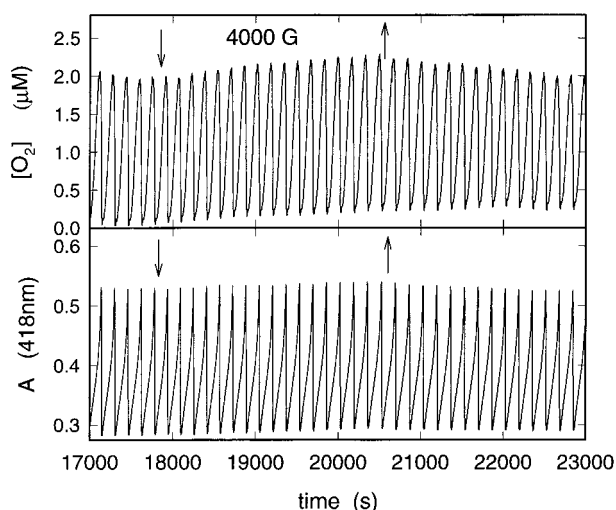


Figure 4. Slow increase in O_2 -oscillation amplitude at a magnetic flux density of 4000 G, owing to an increase in the oxygen-transfer constant.

oscillations. A slight decrease in amplitude of the compound III oscillations is also observed for the 500 G field strength. The difference in response of the oscillations of O_2 and compound III is due to the fact that there is a hyperbolic relation between the amplitude of compound III oscillations and the amplitude of O_2 oscillations: Magnetic perturbations of oscillations where the amplitude of O_2 oscillations is 1 μM or less revealed a much more pronounced decrease in the amplitude of compound III oscillations. Figure 3 shows a plot of the relative change in O_2 oscillation amplitude against the magnetic flux density. We note that the plot is biphasic with a minimum around 1500 G. The increase in oscillation amplitude at field strengths above 3000 G can be ascribed to the increase in oxygen-transfer constant. Figure 4 shows the effect on oscillations when applying a magnetic field of 4000 G. We note that the change in oscillation amplitude is much slower than those observed in Figure 2. The rise in oscillation amplitude occurs in the same time scale as if we had increased the oxygen-transfer constant by increasing the stirring rate. A change in the rate of inflow of NADH induces a slow change in oscillation amplitude similar to that observed in Figure 4. Thus, we can exclude that the effect of magnetic fields of field strength less than 3000 G on oscillation amplitudes is due to an effect on the inflow of O_2 or NADH, and hence it must be an effect on one or more elementary reactions of the reaction cycle. We observed a similar effect of magnetic fields of field strengths

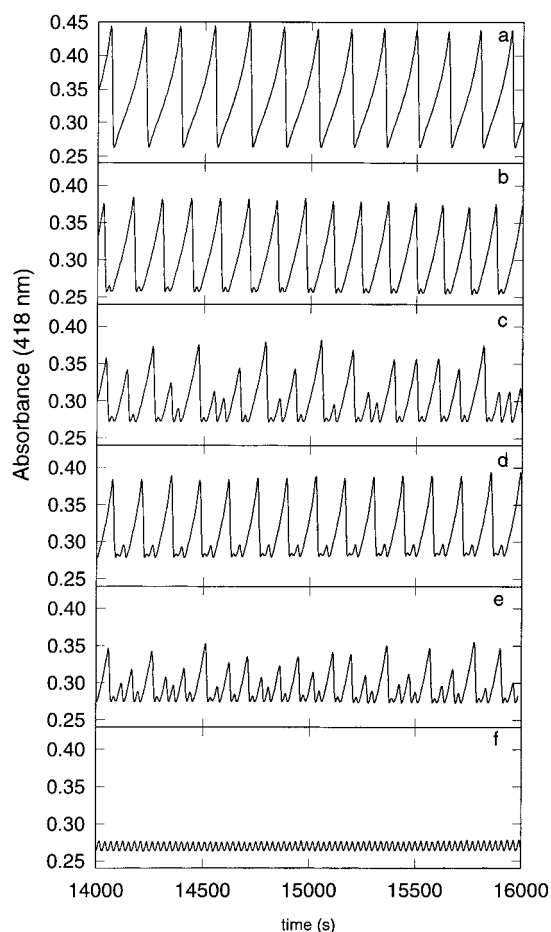


Figure 5. Change in dynamics of the PO reaction as the average NADH concentration is increased. The absorbance of 418 nm measures the concentration of compound III. The experiment was done in the presence of 600 μM 4-hydroxybenzoic acid, and with a background magnetic flux density of 30 G. The average NADH concentrations were (a) 110, (b) 130, (c) 135, (d) 150, (e) 160, and (f) 170 μM . Other experimental conditions as listed in the Experimental Section.

0–3000 G on the oscillation amplitude if 4-hydroxybenzoic acid is replaced by 4-chlorophenol.¹⁴

At concentrations of 4-hydroxybenzoic acid larger than 200 μM , the PO reaction shows complex dynamics.¹⁷ At pH 6.3 such dynamics include mixed-mode oscillations of the type L^S , where L indicates the number of large-amplitude oscillations and S indicates the number of small-amplitude oscillations per period, and chaos. An example is shown in Figure 5 for a concentration of 4-hydroxybenzoic acid of 600 μM . Here the transition from simple periodic oscillations to complex dynamics is induced by changing the mean concentration of NADH, which again is determined by the NADH flow rate. At low concentrations of NADH the dynamics correspond to simple periodic (1^0) oscillations (Figure 5a). As the concentration of NADH is increased further, one observes first a 1^1 oscillation (Figure 5b), then a chaotic state, which we shall label C1 (Figure 5c), then a 1^2 oscillation (Figure 5d). At NADH concentrations higher than those corresponding to the 1^2 state, one observes first a low-amplitude chaotic state (C2, Figure 5e), then a low-amplitude 0^2 oscillation (not shown), and finally a low-amplitude 0^1 oscillation (Figure 5f). Thus the sequence of dynamic states at increasing mean NADH concentration (flow rate) can be listed as $1^0 \rightarrow 1^1 \rightarrow C1 \rightarrow 1^2 \rightarrow C2 \rightarrow 0^2 \rightarrow 0^1$. Similar sequences of dynamic states have been observed following addition of other

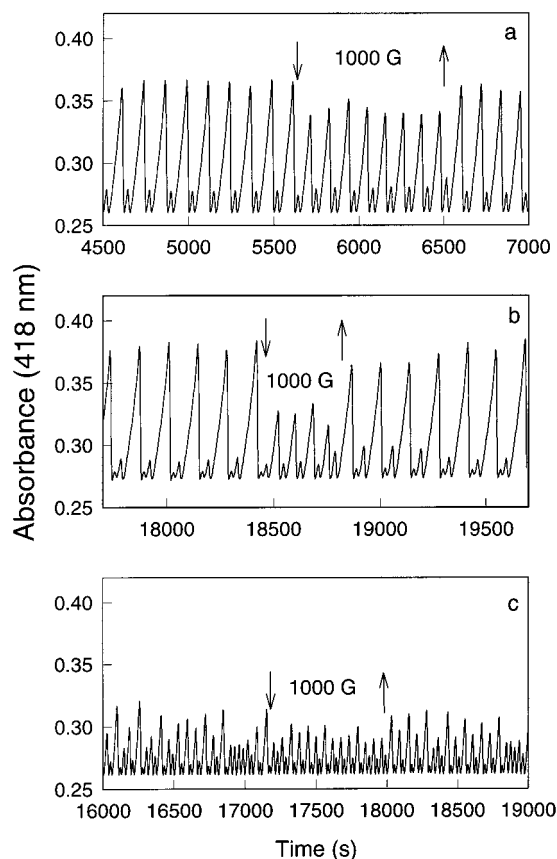


Figure 6. Effect of a magnetic flux density of 1000 G on the dynamics of the PO reaction. The experiments were made in the presence of 600 μM 4-hydroxybenzoic acid. At the time marked \downarrow the magnetic field was turned on, and at the time marked \uparrow the field was turned off. The average NADH concentrations were (a) 129, (b) 144, and (c) 156 μM .

phenolic compounds such as 4-chlorophenol¹⁴ or 2,4-dichlorophenol²⁰ to the reaction system instead of 4-hydroxybenzoic acid.

Some examples of the effect of magnetic fields on complex dynamics in the PO reaction are shown in Figures 6 and 7. The results shown in Figure 6 are in the presence of 4-hydroxybenzoic acid, while the results shown in Figure 7 are in the presence of 4-chlorophenol. In Figure 6 we show that a magnetic field of the order of 1000 G changes the dynamics of a 1^1 periodic state to a 1^1 state of smaller amplitude (Figure 6a). The same magnetic field strength is seen to induce a transition of a 1^2 periodic state to a 0^2 periodic state (Figure 6b) and a small-amplitude chaotic oscillation to a 0^2 periodic state (Figure 6c). In Figure 7a we show a magnetically induced transition from a 1^1 periodic state to its period-doubled (2^2) state. In Figure 7b the magnetic field induces a change from a 1^2 state to its period-doubled (2^4) state, and in Figure 7c the magnetic field induces a change from a 0^2 state to a 0^1 state (inverse period-doubling). Thus, the effect of a magnetic field of the order of 250–1500 G is generally to induce a transition in dynamics from one state to its neighboring state. However, the change in dynamics depends on the size of parameter interval available for a particular dynamic state and its neighboring states. If the parameter interval is very broad, there is no qualitative change in dynamics (Figure 6a). On the other hand, if the system is close to a bifurcation point, only a small magnetic field strength is needed to change the dynamics from one state to a neighboring state (Figure 7b). Sometimes this neighboring state exists only in a narrow parameter interval in which case the magnetic field may induce a shift in the dynamics to the next state over.

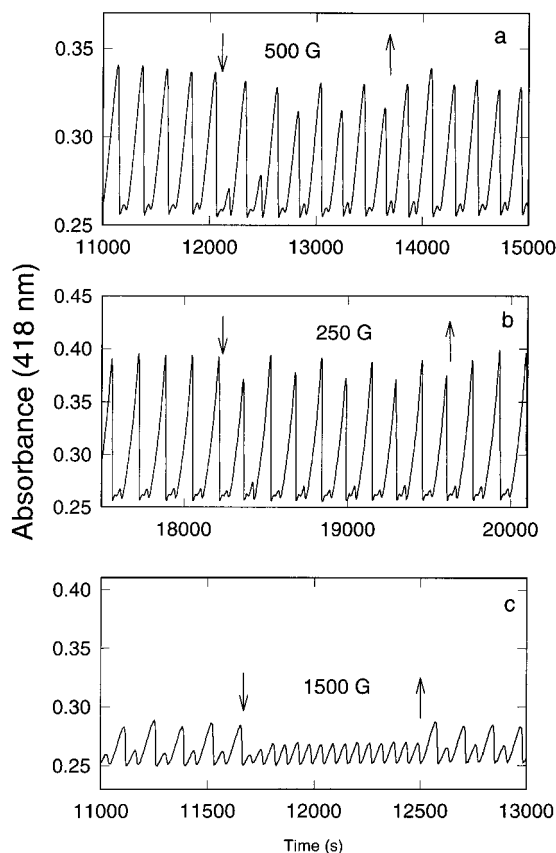
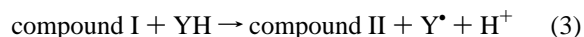


Figure 7. Effect of magnetic flux densities of 250–1500 G on the dynamics of the PO reaction. The experiments were made in the presence of 90 μM 4-chlorophenol. At the time marked \downarrow the magnetic field was turned on, and at the time marked \uparrow the field was turned off. The average NADH concentrations were (a) 126, (b) 150, and (c) 169 μM .

The latter is illustrated in Figure 6b where the magnetic field shifts the dynamics from the 1^2 state to the 0^2 state, thus surpassing the intermediate small-amplitude chaotic state. Thus, the magnetically induced transitions in dynamics seem to depend both on the proximity of the system to a bifurcation point and on the strength of the magnetic field.

IV. Numerical Simulations

The peroxidase–oxidase reaction involves a number of reactions where free radicals are produced or consumed.¹ Table 1 lists some of the reactions together with their reaction constants. The reactions in Table 1 are also known as the BFSO model.²¹ Several of these reactions may be sensitive to magnetic fields. However, here we concentrate on two reactions for which a magnetic field effect has been demonstrated.¹¹ The reactions in question are



where YH is an electron donor (NADH in the present case). Using 2-methyl-1-((trimethylsilyl)oxy)-1-propene as the electron donor, Taraban et al.¹¹ showed up to 15 and 35% reductions in rate of these two reactions when magnetic fields of the order of 500–1000 G were applied. It therefore seems natural to test if such reductions in rates of the two reactions could also explain the effects of magnetic fields on the PO reaction. In order to simplify the simulations and to bring them in accordance with our experimental protocol, we omit the NADH infusion reaction

TABLE 1: Elementary Steps in PO Reaction^a

reaction	$R_i =$	constant
1. $\text{NADH} + \text{O}_2 + \text{H}^+ \xrightarrow{k_1} \text{NAD}^+ + \text{H}_2\text{O}_2$	$k_1[\text{NADH}][\text{O}_2]$	$k_1 = 7 \text{ M}^{-1} \text{ s}^{-1}$
2. $\text{H}_2\text{O}_2 + \text{Per}^{3+} \xrightarrow{k_2} \text{coI}$	$k_2[\text{H}_2\text{O}_2][\text{Per}^{3+}]$	$k_2 = 1.8 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
3. $\text{coI} + \text{NADH} \xrightarrow{k_3} \text{coII} + \text{NAD}^*$	$k_3[\text{coI}][\text{NADH}]$	$k_3 = 4.0 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$
4. $\text{coII} + \text{NADH} \xrightarrow{k_4} \text{Per}^{3+} + \text{NAD}^*$	$k_4[\text{coII}][\text{NADH}]$	$k_4 = 2.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$
5. $\text{NAD}^* + \text{O}_2 \xrightarrow{k_5} \text{NAD}^+ + \text{O}_2^-$	$k_5[\text{NAD}^*][\text{O}_2]$	$k_5 = 2.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
6. $\text{O}_2^- + \text{Per}^{3+} \xrightarrow{k_6} \text{coIII}$	$k_6[\text{O}_2^-][\text{Per}^{3+}]$	$k_6 = 1.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
7. $2\text{O}_2^- + 2\text{H}^+ \xrightarrow{k_7} \text{H}_2\text{O}_2 + \text{O}_2$	$k_7[\text{O}_2^-]^2$	$k_7 = 2.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
8. $\text{coIII} + \text{NAD}^* \xrightarrow{k_8} \text{coI} + \text{NAD}^+$	$k_8[\text{coIII}][\text{NAD}^*]$	$k_8 = 6.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
9. $2\text{NAD}^* \xrightarrow{k_9} (\text{NAD})_2$	$k_9[\text{NAD}^*]^2$	$k_9 = 8.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$
10. $\text{Per}^{3+} + \text{NAD}^* \xrightarrow{k_{10}} \text{Per}^{2+} + \text{NAD}^+$	$k_{10}[\text{Per}^{3+}][\text{NAD}^*]$	$k_{10} = 1.8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$
11. $\text{Per}^{2+} + \text{O}_2 \xrightarrow{k_{11}} \text{coIII}$	$k_{11}[\text{Per}^{2+}][\text{O}_2]$	$k_{11} = 1.0 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$
12. $\text{NADH}(\text{stock}) \xrightarrow{k_{12}} \text{NADH}(\text{liquid})$	$k_{12}[\text{NADH}]_{\text{st}}$	$k_{12} = \text{variable}$
13. $\text{O}_2(\text{gas}) \xrightleftharpoons[k_{-13}]{k_{13}} \text{O}_2(\text{liquid})$	$k_{13}[\text{O}_2]_{\text{eq}} = 7.2 \times 10^{-8} \text{ M s}^{-1}$ $k_{-13}[\text{O}_2]$	$k_{-13} = 6.0 \times 10^{-3} \text{ s}^{-1}$

^a The different oxidation steps of the enzyme are ferric peroxidase (Per^{3+}), ferrous peroxidase (Per^{2+}), compound I (coI), compound II (coII), and compound III (coIII). For the corresponding differential equations, see ref 20.

(reaction 12 in Table 1) from the model and treat the concentration of NADH as a constant instead of as a variable. Thus, instead of the NADH infusion rate^{9,20,22} we may use the NADH concentration as a bifurcation parameter as in the experiments. Furthermore, the number of differential equations to be solved decreases from 10 to 9. A few test simulations showed that the effect of turning NADH into a constant had relatively little effect on the dynamics of the BFSO model. This observation is in good agreement with the earlier findings that NADH is a nonessential variable.^{15,23–26}

Figure 8 shows the dynamics of compound III for different concentrations of NADH. We note that apart from the simple periodic oscillations, which are observed at unrealistic low concentrations of NADH, there is good qualitative and quantitative agreement between the simulations and the experimental observations shown in Figure 5. Figure 9 shows a bifurcation diagram of the dynamics versus the NADH concentration. Here amplitudes of compound III oscillations are plotted as a function of the NADH concentration. We note that the order of bifurcations is the following: $1^0 \rightarrow 1^1 \rightarrow \text{C1} \rightarrow 1^2 \rightarrow \text{C2} \rightarrow 1^3 \rightarrow \text{C3} \rightarrow 0^2 \rightarrow 0^1$; i.e., there is one more mixed-mode oscillatory state (1^3) and one more chaotic state (C3) as compared to the experimentally observed states.

To probe the effect of the above hypothesis that magnetic fields between 0 and 3000 G decrease the rate constants k_3 and k_4 , we adjusted the NADH concentration to the various types of dynamics in the bifurcation diagram and then decreased one or both of these rate constants. The results are shown in Figures 10 and 11. We note that by decreasing these rate constants by a few percent we are able to simulate all the experimentally observed effects of magnetic fields shown in Figures 6 and 7. An alternative to the simulation in Figure 10c is presented in Figure 12a. Here we show that a decrease of k_4 of as little as 4% changes the dynamics from a large-amplitude chaotic state to a small-amplitude chaotic state as evidenced by the next-amplitude plots in Figure 12b. Figure 13 shows the corresponding experimental next-amplitude plots of the time series from Figure 6c before and during the application of the magnetic field.

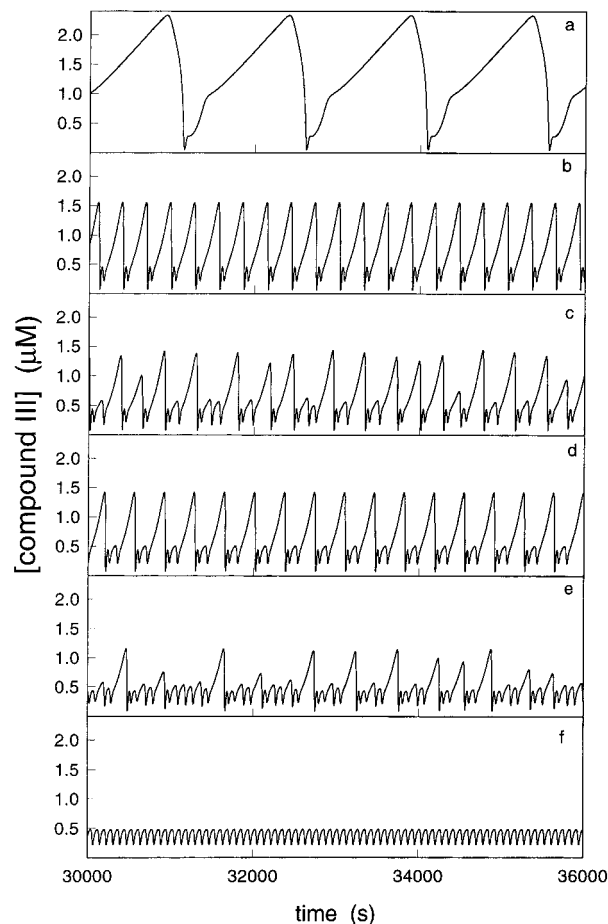


Figure 8. Dynamics of the modified BFSO model at different constant NADH concentrations: (a) 10, (b) 110, (c) 121, (d) 122, (e) 132.3, and (f) 138 μM . Rate constants as listed in Table 1. Initial conditions: $[\text{Per}^{3+}]_0 = 2.4 \mu\text{M}$, while all other initial concentrations were set to 0.

Discussion

We have shown that magnetic fields affect the dynamics of the oscillating PO reaction. Both simple periodic oscillations and complex dynamics can be perturbed. The biphasic response of oscillation amplitude to changes in magnetic flux density is

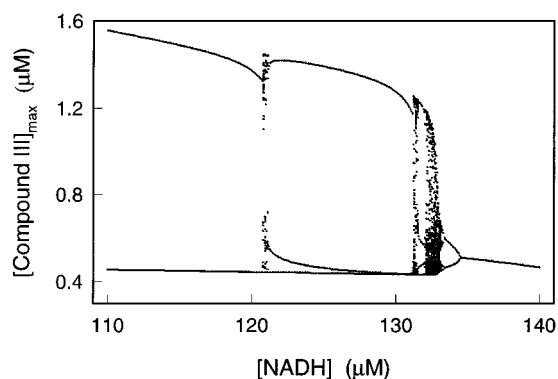


Figure 9. Bifurcation diagram of the modified BFSO model. Amplitudes of compound III oscillations are plotted at different constant NADH concentrations. Rate constants and initial conditions as in Figure 8.

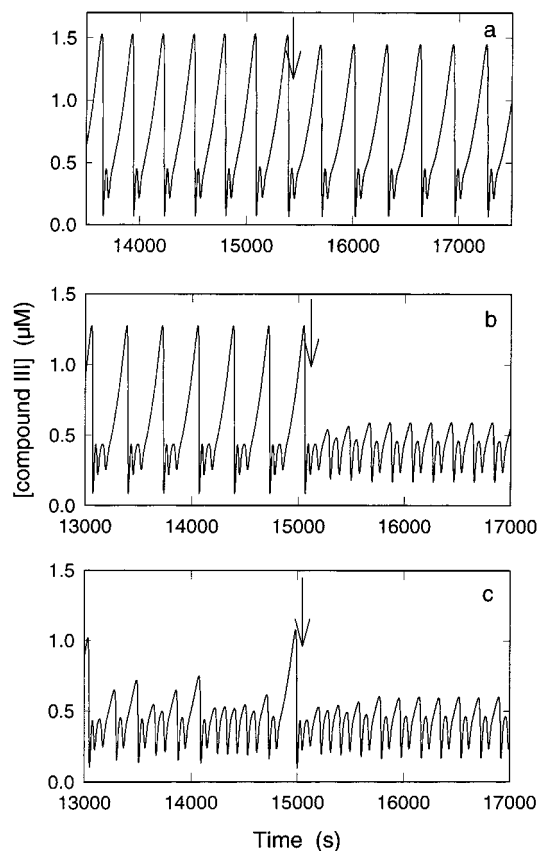


Figure 10. Effect of changing k_3 and k_4 on the dynamics of the modified BFSO model. (a) The concentration of NADH is 112 μM ; at the time indicated by \downarrow k_3 was reduced from $4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $3.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ while k_4 was reduced from $2.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$. (b) The concentration of NADH is 130 μM ; at the time indicated by \downarrow k_3 was reduced from $4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $3.9 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ while k_4 was reduced from $2.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $2.2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$. (c) The concentration of NADH is 132.45 μM ; at the time indicated by \downarrow k_3 was reduced from $4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $3.95 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ while k_4 was reduced from $2.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $2.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$.

very similar to the changes in rate observed for reactions involving a radical pair mechanism.¹² As for which processes are affected by the magnetic field, we have proposed the reactions 3 and 4 in Table 1, because these reactions are known to involve a radical pair mechanism and they have previously been shown to be affected by a magnetic field.¹¹ Simulations of a model of the PO reaction support this hypothesis, but other reactions in the reaction cycle may be affected as well. Our simulations of the effect of magnetic fields will qualitatively

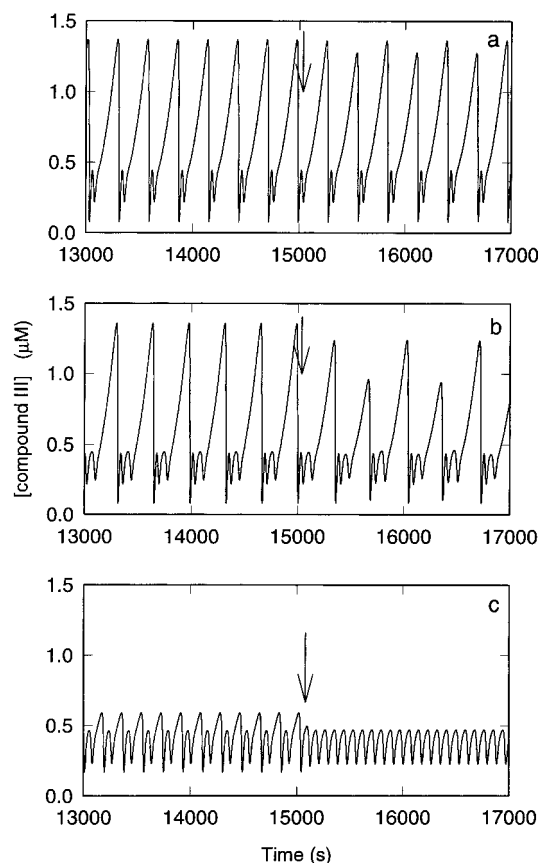


Figure 11. Effect of changing k_3 and k_4 on the dynamics of the modified BFSO model. (a) The concentration of NADH is 120 μM ; at the time indicated by \downarrow k_4 was reduced from $2.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $2.46 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$. (b) The concentration of NADH is 127 μM ; at the time indicated by \downarrow k_3 was reduced from $4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $3.9 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ while k_4 was reduced from $2.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$. (c) The concentration of NADH is 133.5 μM ; at the time indicated by \downarrow k_3 was reduced from $4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $3.9 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ while k_4 was reduced from $2.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $2 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$.

reproduce the effects of the magnetic field. However, the simulations do not provide a quantitative correspondence between the experimentally observed effect of magnetic flux density and the effect of changing the rate constants k_3 and k_4 . There may be several possible explanations of this fact. One possibility is that the modified BFSO model does not reproduce correctly the chemistry of the PO reaction. This possibility is not very likely since the model can reproduce most other observed features of the experimental system.¹ Another possibility is that one or more of the rate constants used in the simulations is not correct. Most of the rate constants in the reaction are not known with a high degree of accuracy,¹ and changing one rate constant may result in small changes in the sequence of periodic and chaotic states in the bifurcation diagram and the size of the parameter interval in which these states occur.^{9,10,20,22} A further complication of the experimental system is that once the system has been placed in a particular dynamic state, experimental precision does not allow us to decide precisely where the system is in the parameter interval, e.g., how close the system is to the boundary of a neighboring state. Therefore, two experiments where the same magnetic flux is applied to the same dynamic state may well give two slightly different results. Thus, we think that the BFSO model accounts fairly well for the effects of the magnetic field on the PO reaction.

The present finding and the previous demonstrations^{11,12,14} that magnetic fields may change the kinetics of reactions

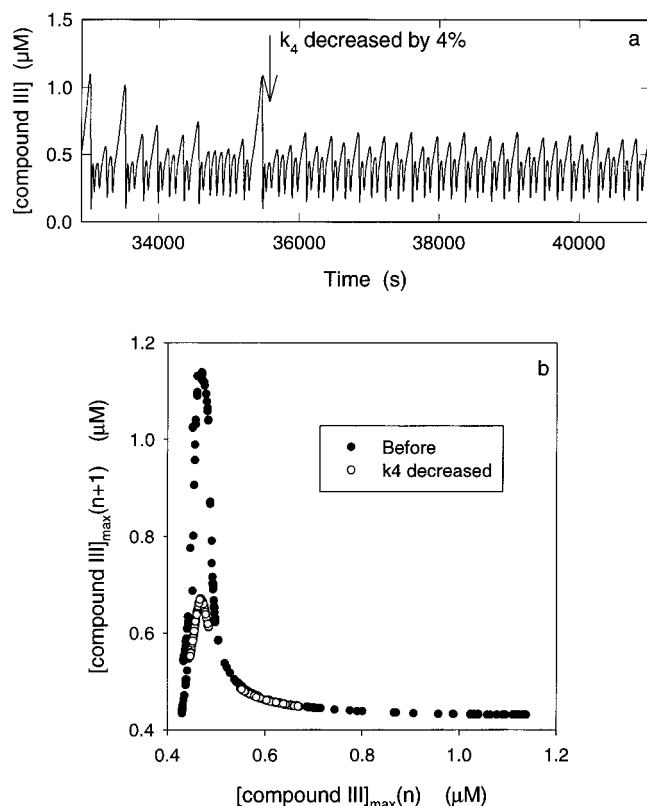


Figure 12. Effect of changing k_3 and k_4 on the dynamics of the modified BFSO model. (a) The concentration of NADH is $132.45 \mu\text{M}$; at the time indicated by \downarrow k_4 was reduced from $2.6 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ to $2.5 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$. (b) Next amplitude plot of the oscillations of compound III before (\bullet) and after (\circ) the decrease in k_4 .

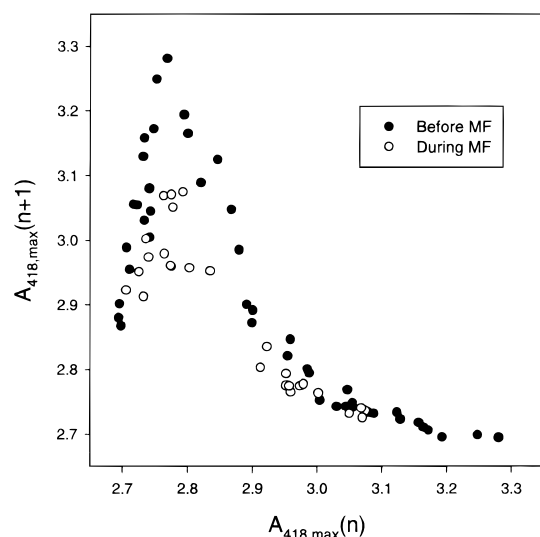


Figure 13. Next-amplitude plot of the oscillations of compound III from Figure 6c before and during the application of a magnetic field of 1000 G.

catalyzed by enzymes containing transition metals have large implications for our view on the effect of magnetic fields on biological material in general. Many signal-transduction cascades in biology involve transition metals.²⁷ Furthermore, signal-transduction cascades often encode the information in oscillating signals,²⁸ and these sometimes have highly complex periods.^{29,30}

If one or more reactions in a signal-transduction cascade is changed by only a few percent, it will have tremendous effects on the information conveyed by the cascade, and one could easily imagine that many of the difficulties in exactly reproducing the effect of a magnetic field on a biological process in different laboratories are due to the fact that small differences in experimental protocols will be magnified through the nonlinearities of the system and lead to completely different outcomes.

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