# Effect of Magnetic Fields on an Oscillating Enzyme Reaction
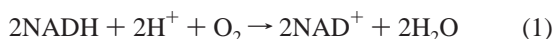
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*Received March 15, 1999*

**Abstract:** Magnetic fields were found to have a profound effect on the dynamics of the oscillating peroxidase−oxidase (PO) reaction. Static magnetic fields up to 3000 G were applied to this biochemical reaction, and their effects on the oscillatory dynamics were investigated. When the system is in a simple periodic state, magnetic fields induce a decrease in the oscillation amplitude. When the system is in a state of complex dynamics magnetic fields may induce a shift in dynamics to a neighboring state. Thus, magnetic fields may be used for noninvasive manipulations and control of complex dynamics.

## Introduction

While it is well-established that magnetic fields influence biological processes involving photochemical reactions[1] the magnetic field dependence of biological activity in general is still somewhat controversial.[2,3] Nevertheless, a handful of enzyme systems are known where magnetic field-induced changes in reaction rates have been measured and for which a molecular mechanism for the effect of the magnetic field can be established.[2] Most of these systems seem to involve a radical pair mechanism, i.e., a reaction where a pair of free radicals is either generated or consumed. The origin of the effect of a magnetic field lies in the influence of the field on the interconversion of singlet and triplet spin states of the radical pair.[4] The fact that some enzyme-catalyzed reactions are sensitive to magnetic fields opens up a new possibility of altering the course and the kinetics of such reactions. One advantage to using a magnetic field to perturb biochemical reactions is that this can be done noninvasively, and hence perturbations can be applied also to the system in the intact cell. It would therefore be of general interest to find relatively simple enzyme systems whose kinetics can be manipulated using magnetic fields. Here we present such a system: the oscillating peroxidase−oxidase (PO) reaction.[5] The PO reaction involves the oxidation of reduced nicotinamide adenine dinucleotide (NADH) by molecular oxygen

$$2NADH + 2H^+ + O_2 \rightarrow 2NAD^+ + 2H_2O \qquad (1)$$

The reaction is catalyzed by peroxidases from different sources.[6] In the laboratory peroxidase from horseradish roots is the most commonly used enzyme. The PO reaction is stimulated by various phenolic compounds and aromatic amines and inhibited by methylene blue.[7] When NADH and $O_2$ are supplied continuously to a well-stirred homogeneous solution containing peroxidase, a suitable phenol and methylene blue, the concentrations of the substrates and various enzyme intermediates oscillate. Both simple periodic oscillations and complex dynamics have been observed.[8] It was recently suggested from theoretical studies that the oscillations in the PO reaction may be affected by magnetic fields.[9] Here we report experimental verification of these predictions.

## Experimental Section

Experiments were performed in a 20 mm × 20 mm × 43.5 mm quartz cuvette fitted with a thermostating jacket and a stirring motor mounted above the cuvette. The motor was connected through a stirring shaft to a stirring propeller situated approximately 1 mm above the bottom of the quartz cuvette. To ensure rapid mixing and homogeneity a stirring rate of 1200 rpm was used. The experimental system was mounted between the iron poles of a 4 in. electromagnet (Walker Scientific Inc., Worcester, Massachussets). The background magnetic flux arising from the iron poles is 30 G. In all of the experiments where magnetic fields were applied, the field returned to a value of 25 to 35 G after switching the current to the electromagnet off. The static magnetic flux was measured in the middle of the cuvette with a Hall probe connected to a Gaussmeter (F. W. Bell, Orlando, Florida). The setup was connected to a Zeiss Specord S10 diode array spectrophotometer through optical fibers. The absorbances in the range 350−600 nm (1 nm resolution) were recorded together with the $O_2$ concentration and stored in a computer for later analysis. The liquid sample consists of 8 mL of 100 mM sodium phosphate buffer, pH 6.3, containing 2.4 $\mu$M horseradish peroxidase, 30−90 $\mu$M 4-chlorophenol, and 0.1 $\mu$M methylene blue. The temperature of the sample was 28 (± 0.1) °C.

Oxygen was supplied as a partially moistured mixture of $O_2$ and $N_2$ corresponding to 1.05% (v/v) $O_2$. The gas mixture was fed to a 9.3

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mL gas headspace above the liquid. The rate of diffusion of $O_2$ into the liquid is determined by the equation[10]

$$v_{O_2} = k_t([O_2]_{eq} - [O_2]) \tag{2}$$

where $[O_2]_{eq}$ is the oxygen concentration in the liquid at equilibrium between the gas phase and the liquid, $[O_2]$ is the actual oxygen concentration in the liquid, and $k_t$ is the oxygen-transfer constant. The magnitude of $k_t$ depends on the temperature and the surface area of the gas−liquid interface and hence on the stirring rate. $k_t$ was measured as $(5.8 \pm 0.2) \times 10^{-3}$ s$^{-1}$. Oxygen in the sample was measured with a Clark electrode (Microelectrodes Inc., Bedford, New Hampshire). NADH was supplied as a 0.1 M aqueous solution in distilled water through a capillary whose tip was below the surface of the solution. The capillary was connected to a syringe pump (Harvard Apparatus, model 22). The inflow rate of NADH solution was between 40 and 55 $\mu$L/h. NADH was measured as the absorbance at 370 nm.

The reactor containing the solution of enzyme, methylene blue, and 4-chlorophenol in phosphate buffer was equilibrated with pure nitrogen before the start of an experiment. Experiments were typically started by adding NADH at a flow rate of 50 $\mu$L/h. As the absorbance at 370 nm reached an OD of 0.6−0.7, the composition of the gas stream was switched from pure $N_2$ to the $O_2$−$N_2$ mixture. The NADH flow rate was then adjusted such that the NADH concentration oscillated around a constant level. By choosing different flow rates we were able to stabilize the NADH concentration on different values corresponding to different types of dynamics.[7]

## Results and Discussion

Different types of oscillatory behavior may be observed in a semibatch reactor, i.e., a reactor to which substrates are supplied continuously, but neither excess liquid nor products are removed. Figure 1 depicts oscillations in the concentration of dissolved oxygen in the PO reaction in a semibatch reactor under different experimental conditions. Figure 1A through E illustrate how the dynamics change as the rate of inflow of NADH is increased. At low inflow rates only simple periodic oscillations are observed. As the inflow rate is increased, more complex oscillatory patterns are obtained. We shall characterize periodic oscillations using the symbolic notation $L^S$ where $L$ indicates the number of large-amplitude oscillations and $S$ indicates the number of small-amplitude oscillations per period. The periodic oscillations shown in Figure 1A through C are thus to be labeled $1^0$, $1^1$, and $1^2$, respectively. The oscillations observed in Figure 1D represent a deterministic chaotic state which we shall label "C", whereas the small-amplitude periodic oscillations in Figure 1E are labeled $0^1$. Between the chaotic state C and the $0^1$ periodic state we observe a $0^2$ periodic state (not shown). Thus, in the presence of 4-chlorophenol the sequence of oscillatory and chaotic states following an increase in flow rate of NADH can roughly be ordered as follows: $1^0 \rightarrow 1^1 \rightarrow 1^2 \rightarrow ... \rightarrow C \rightarrow 0^2 \rightarrow 0^1$.[11] The experimentally observed states following an increase in NADH inflow rate have been simulated with a detailed model of the PO reaction.[12]

To study the effect of magnetic fields on the oscillatory dynamics of the PO reaction the experimental setup was mounted between the poles of a 4 in. electromagnet. Figure 2 shows the effect on simple periodic ($1^0$) oscillations of changing the magnetic field strength from the 30 G background to field
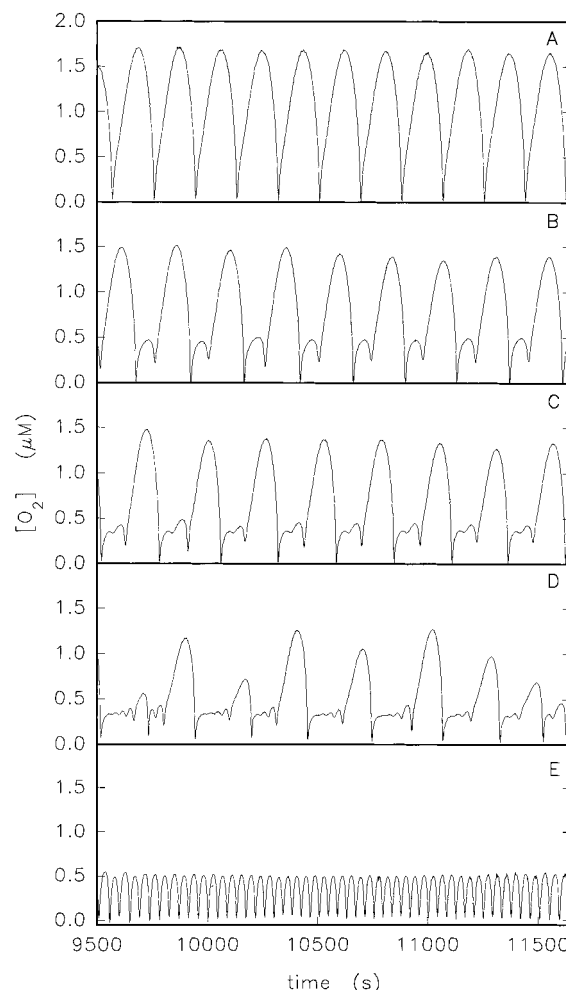
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(11) More periodic states than those shown here have been observed experimentally in the presence of 2,4-dichlorophenol instead of, as here, 4-chlorophenol. These include $1^3$ and $1^4$ and concatenated states such as $1^3$ $1^4$ and period-doubled states $2^0$ and $2^2$. [Hauser, M. J. B.; Olsen, L. F. *J. Chem. Soc., Faraday Trans.* **1996**, *92*, 2857−2863].

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**Figure 1.** Oscillatory dynamics of dissolved oxygen in the PO reaction at increasing flow rate of NADH. The reaction mixture contained 2.4 $\mu$M peroxidase, 60 $\mu$M 4- chlorophenol, and 0.1 $\mu$M methylene blue. The inflow rate of NADH solution was: (A) 42 $\mu$L/h; (B) 43 $\mu$L/h; (C) 45 $\mu$L/h; (D) 48 $\mu$L/h; (E) 50 $\mu$L/h. The temperature was 28 °C. The experiments were performed on a background magnetic flux of 30 G as explained in the Experimental section. Other experimental conditions are as described in the Experimental Section

strengths of 250 and 750 G, respectively. We note that the field induces a rapid and reversible decrease in the oscillation amplitude of $O_2$. The decrease in amplitude becomes more pronounced with increasing field strength.[13] Such a decrease in oscillation amplitude implies an increase in the average rate of consumption of $O_2$ and hence also an increase in the oxidation rate of NADH. However, the effects described above could also be caused by changes in experimental conditions such as an increase in temperature, an increase in the flow rate of NADH, or a decrease in the oxygen transfer constant $k_t$. To exclude such effects we measured the influence of magnetic fields on the temperature in the liquid and on the oxygen-transfer constant. The temperature remained within the limits of resolution of our thermocouple (0.1 °C) before, during, and after the application of the field. Likewise, we were unable to measure a change in stirring rate when switching on a magnetic field in the range 30−3000 G. Finally, we measured the oxygen-transfer constant before, during, and after the application of a magnetic field up

(13) In fact a biphasic response is observed. Magnetic field strengths up to about 1500 G induce an increasing decline in oscillation amplitude. At field strengths greater than 1500 G the decline in amplitude diminishes, and at field strengths over 4000 G an increase in amplitude is observed. A part of this increase is due to a magnetically induced increase in $k_t$.
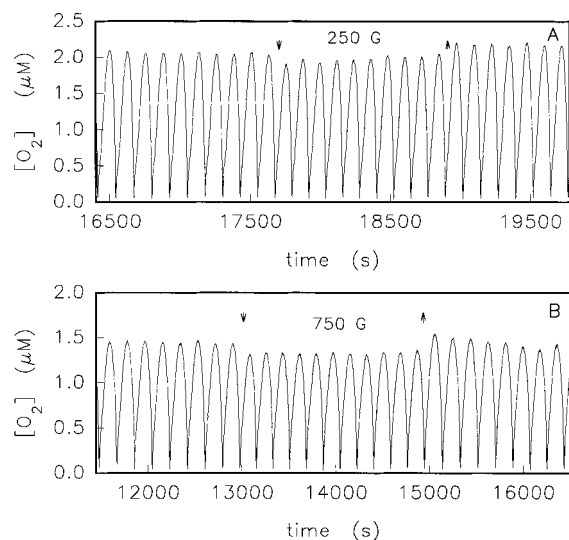
**Figure 2.** Effect of static magnetic field on simple periodic oscillations in the PO reaction. The experimental conditions were the same as in Figure 1 except that only 30 $\mu$M 4-chlorophenol was present in the sample. NADH infusion rate was 44 $\mu$L/h. At times marked ↓ the magnetic flux density was increased from the background of 30 G to 250 G (A) and 750 G (B), respectively. At times marked ↑ the magnetic flux density was again decreased to the 30 G background.

to 3000 G. In all these measurements $k_t$ remained within experimental error ($\pm 0.2 \times 10^{-3}$ s$^{-1}$). At higher field strengths (between 3000 and 5000 G) a slight *increase* in $k_t$ was observed. However, an increase in $k_t$ would result in an increase in oscillation amplitude, i.e., the opposite effect to that observed in Figure 2. We also studied the effect of changing the temperature, the flow rate of NADH, and the oxygen-transfer constant $k_t$ on the oscillations. In all cases an immediate change in any of these parameters resulted in a much slower change in oscillation amplitude compared to those seen in Figure 2. Thus, we can exclude that the magnetic field-induced decrease in amplitude is due to changes in temperature, NADH flow rate, or $k_t$.

Next, we investigated the effect of magnetic fields on the complex dynamic states observed in the PO reaction. Some examples are presented in Figure 3. Figure 3A shows that a magnetic flux density of 500 G may induce a shift in dynamics from a 1$^1$ periodic state to its period-doubled state 2$^2$. A field of similar flux density may induce a shift in dynamics from a chaotic state to a 1$^2$ state as illustrated in Figure 3B or a shift from a 0$^2$ oscillation to a 0$^1$ oscillation as shown in Figure 3C. Thus, the effect of a magnetic field of the order of 500 G is generally to shift the dynamics of the PO reaction from one state to a neighboring state. Applications of magnetic flux densities of 1000 G or more induce larger shifts in dynamics, e.g. a shift in dynamics from a 1$^1$ periodic state to a 1$^2$ periodic state. Magnetic flux densities of 250 G or less induce only a transient change in dynamics, e.g., a transient change of the 1$^1$ to a 2$^2$ state that slowly reverts to the 1$^1$ state. These and other experiments allow us to propose a sequence of dynamic states as: 1$^0 \rightarrow$ 1$^1 \rightarrow$ 2$^2 \rightarrow$ C$_1 \rightarrow$ 1$^2 \rightarrow$ ... $\rightarrow$ C$_2 \rightarrow$ 0$^2 \rightarrow$ 0$^1$. Hence the magnetic field experiments have revealed two additional states which are extremely difficult to observe using traditional methods of perturbation as they exist only in a narrow interval of parameter space. These are the period-doubled state 2$^2$ and the chaotic state C$_1$.[11] To ensure that the perturbation of the chaotic state in Figure 3B is reversible we computed next-amplitude plots of the aperiodic fluctuations in oxygen concentrations before the application of the magnetic field and after
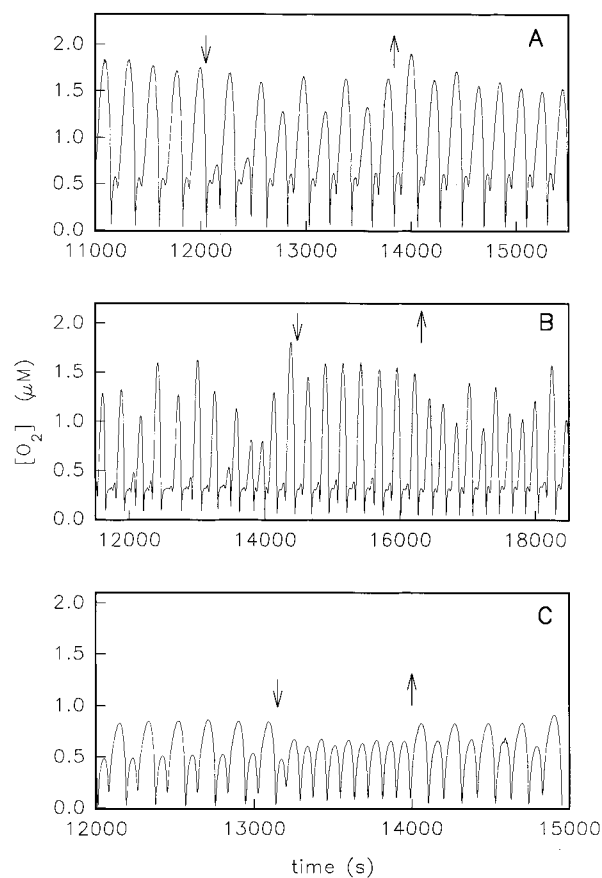


**Figure 3.** Effect of static magnetic field on complex periodic oscillations and chaos in the PO reaction. Experimental conditions as in Figure 1, except that 90 $\mu$M 4-chlorophenol was present in the sample. The flow rates of NADH were (A) 44 $\mu$L/h, (B) 49 $\mu$L/h and (C) 52 $\mu$L/h. At times marked by ↓ the magnetic flux density was increased from the background of 30 G to 500 G, and at times marked ↑ the magnetic flux density was again decreased to the 30 G background.
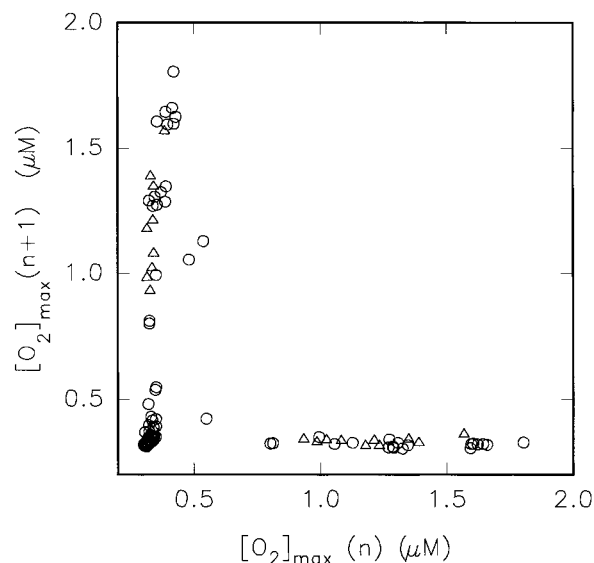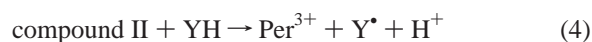


**Figure 4.** Next-amplitude plot of the aperiodic oscillations from Figure 3B: (△) before the 500 G magnetic field is turned on, (○) after the 500 G magnetic field is turned off.

the field was switched off. Both plots yield points superimposed on the same almost one-dimensional one-humped map from which chaos in the data can be inferred (Figure 4). Thus, after the field is switched off, the dynamics return to the same chaotic state as before the field was applied. As for the perturbations

of the $1^1$ oscillation in Figure 3A and the $0^2$ oscillation in Figure 3C a short transient is observed after switching the magnetic field on. During this transient the system gradually converges on its asymptotic state.

The results described above suggest at least three potential applications of perturbations of an oscillating reaction using magnetic fields. First, the perturbations may be used to establish the order in which nearby dynamic states occur. Second, transients that occur during the transition from one state to the other can provide us with important information that may be used in establishing the reaction mechanism.[14,15] Finally, the techniques to control complex dynamics in chemical reactions[16,17] can be carried over to magnetic fields. A time-varying magnetic field presents a rapid noninvasive technique to control oscillations and complex dynamics. Thus, perturbations can also be applied to the PO reaction in intact cells.

The PO reaction encompasses a minimum of 10−15 elementary reactions, several of which involve the formation or consumption of free radicals.[5] It is therefore tempting to conclude that one or more reactions involving a radical pair mechanism[2] may be responsible for the reaction's magnetosensitivity. This conclusion is further supported by the biphasic response of the change in oscillation amplitude[13] which is typical of radical pair mechanisms.[1,2] Although the exact reaction step(s) cannot be disclosed at this point, our attention is drawn to two reaction steps for which a radical pair mechanism has recently been established.[18] These steps are the oxidations of an electron donor by the peroxidase intermediates compound I and compound II

$$\text{compound I} + \text{YH} \rightarrow \text{compound II} + \text{Y}^\bullet + \text{H}^+ \quad (3)$$

$$\text{compound II} + \text{YH} \rightarrow \text{Per}^{3+} + \text{Y}^\bullet + \text{H}^+ \quad (4)$$

where YH and $\text{Per}^{3+}$ denote the electron donor and native ferric peroxidase, respectively. It was shown that the rate constants of these two reactions decrease by up to 35% in magnetic fields of the order of 500−1000 G when 2-methyl-1-((trimethylsilyl)-oxy)-1-propene was the electron donor.[18] Other recent experiments have indicated that reactions 3 and 4 are essential for the observation of sustained oscillations and complex dynamics in the PO reaction.[19] Since the observation of such dynamics requires the presence of catalytic amounts of a phenolic compound it has been speculated that the role of the phenol is to regulate the oxidation rate of NADH through reactions of compound I and compound II with the phenol (YH).[19] The phenoxyl radical ($\text{Y}^\bullet$) formed in these reactions is reconverted to the phenol through reactions with NADH

$$\text{Y}^\bullet + \text{NADH} \rightarrow \text{YH} + \text{NAD}^\bullet \quad (5)$$
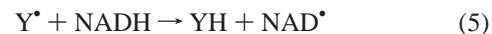
The $\text{NAD}^\bullet$ radical is a key intermediate in the PO reaction. The radical acts as a switch that controls the start and stop of the consumption of NADH and oxygen, thus forming the basis of the reaction's ability to oscillate.[5,12] By using a slight modification of a recent detailed model of the PO reaction[12] the effect of changing the rate constants of reactions 3 and 4 on the oscillations and complex dynamics of the PO reaction was investigated numerically.[20] The numerical simulations showed that a decrease in the rate constant of reaction 4 by a few percent is sufficient to account for many of the magnetic field effects shown in Figures 2 and 3. The simulations are therefore in agreement with the previous experimental observations of magnetic field effects on reactions 3 and 4 using a different hydrogen donor.[18]

The PO reaction occurs in vivo in both plant and animal cells.[21,22] In plant cells $\text{NAD}^+$ formed in reaction 1 is reconverted into NADH through the oxidation of malate. Furthermore, the in vivo reaction also requires the presence of a number of phenolic compounds.[23] This suggests that magnetic fields may be used as a noninvasive tool to control the course and the dynamics of peroxidase−oxidase linked reactions in vivo.

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