

Effects of Periodic and Stochastic Perturbations on Oscillations and Chaos in a Model of the Peroxidase-Oxidase Reaction

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The effects of periodic and random perturbations on the periodic and chaotic oscillations in a model of the peroxidase-oxidase reaction are investigated. The perturbations were chosen to be comparable in size and frequency to those measured in the experimental system. Small periodic perturbations did not affect the dynamics significantly. Small random perturbations, on the other hand, could totally obscure simple periodic dynamics whereas chaotic dynamics turned out to be relatively robust to such perturbations.

1. Introduction

Biological systems are dissipative systems with high capacities for displaying complex dynamic behaviours. This is a manifestation of the non-linearities in their dynamic equations. Even for the simplest systems such equations contain non-linearities that make them analytically unsolvable. Analytic solutions may be obtained following linearisation, but usually this is accompanied by loss of their biological relevance. Non-linear dynamics is the study of motion in such non-linear systems. One of the most recent advances of non-linear dynamics in biology is the establishment of chaotic behaviour as a new dynamic mode, alternative to the classical modes steady state and limit cycle behaviour [1].

Chaotic behaviour may be described as non-periodic cyclic motion with a property known as "sensitivity to initial conditions" [2]. The latter property results in chaotic systems sharing many properties with stochastic systems, and consequently it is often very difficult to decide whether a given process is chaotic or stochastic [3]. Although there are numerical methods available to detect chaos in a given set of data these methods have several shortcomings, in particular when background perturbations are present.

Perturbations are always a problem to the experimentalist and in particular to experimentalists studying chaotic behaviour. Perturbations may be

divided into two subclasses: a) those generated by the measuring system, usually referred to as observational perturbations and b) those imposed on the system from the environment, which we shall call external perturbations [4]. Since most biological systems with chaotic dynamics are open to their surroundings the major source of external perturbations are periodic or random perturbations of the inlets. It is therefore very important to study the dynamics of systems where fluctuations are deliberately added or where the nature and the magnitude of such fluctuations are known. Here I present investigations of fluctuations imposed on a model of the oscillating peroxidase-oxidase reaction which is known to display chaotic motion [5–7]. Two types of fluctuations were applied: Small periodic perturbations and small random fluctuations of the inlets of reactants. The fluctuations were chosen so as to match those that can be detected in the experimental system. The results of these numerical investigations were compared with the results obtained with the experimental system.

2. The Experimental System and Sources of Fluctuations

The peroxidase-oxidase reaction is the peroxidase (EC 1.11.1.7) catalysed oxidation of reduced nicotinamide adenine dinucleotide (NADH) with molecular oxygen as the electron acceptor. When the reaction takes place in an open system where both reactants, NADH and O_2 , are continuously supplied, the concentrations of these will oscillate with

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a mode and frequency that depend on the concentration of peroxidase. At some enzyme concentrations these oscillations are chaotic [5–7].

O₂ is entering the reaction mixture by passive diffusion from a N₂/O₂ gas phase above the rapidly stirred solution through the gas/liquid interface. The rate of diffusion of O₂ into the liquid is given by the rate law

$$r = K([O_2]_{eq} - [O_2]), \quad (1)$$

where [O₂] is the oxygen concentration in the liquid, [O₂]_{eq} is the concentration at equilibrium and *K* is a constant, the value of which depends on the surface area of the gas/liquid interface and the volume of the liquid. Changes in *r* may result from changes in *K* or from changes in the N₂/O₂ composition of the gas phase. NADH is supplied by pumping a concentrated solution into the liquid through a capillary at a constant rate using a high precision syringe pump.

The oscillations of the reaction are of the relaxation type with critical concentrations of NADH and O₂ at which the reaction “turns on” and “turns off” [8]. These circumstances offer the possibility of directly determining the nature and the extent of fluctuations of inlets of reactants since the reaction is only proceeding for a very short time (a few seconds) compared to the average period length of the oscillations (several minutes for one excursion). Figure 1a shows a magnification of the oscillations of NADH in the chaotic state. The increase in NADH is solely due to the pumping and we note small periodic or random fluctuations in the rate of pumping due to imperfections in the pump. Similarly the fluctuations in the diffusion of O₂ can be estimated by plotting the amplitude of the excursions of O₂ against the period length as shown in Figure 1b. Ideally this plot should be defined by the equation

$$[O_2]_{max} = [O_2]_{eq}(\exp(KP) - 1)/\exp(KP), \quad (2)$$

where [O₂]_{max} and *P* denote the amplitude and period of an excursion respectively. Deviations from this curve are due to imperfections in the O₂ inlet resulting from either slow changes in the stirring rate or to changes in the O₂ content of the O₂/N₂ gas phase above the liquid. Other experiments revealed that these changes are almost periodic with periods considerably longer than the periods of oscillations.

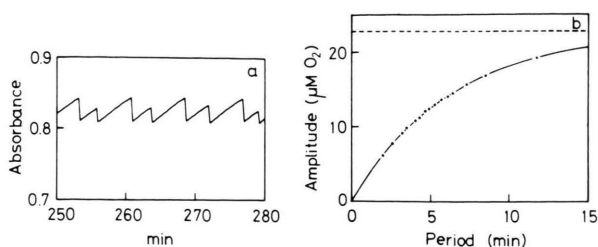


Fig. 1. Estimations of fluctuations of the inlets of NADH and O₂ in the peroxidase-oxidase reaction. a) Chaotic oscillations of NADH measured as the absorbance at 380 nm. Fluctuations in the pumping rate are seen as “wiggles” on the rising parts of the curve. b) Plot of the amplitudes of O₂ oscillations measured with a Clark electrode against the period length in the chaotic state. The solid curve is drawn using (2) and the value of *K* measured in a separate experiment. The experiment was performed in a 4.5 ml hexagonal glass cuvette containing 0.55 μM peroxidase, 0.2 μM methylene blue and 10 μM 2,4-dichlorophenol in 0.1 M Na-acetate, pH 5.1. O₂ in the gas phase was 1.9 per cent (*v/v*). 0.2 M NADH was pumped into the reaction mixture at a rate of 11 μl per hour. Temperature was 28 °C.

3. The Model

Oscillations and chaos in the peroxidase-oxidase reaction can be simulated by a branched chain mechanism involving free radicals, resulting in the following four non-linear differential equations [6, 7]:

$$dA/dt = k_7(A_0 - A) - k_3ABY, \quad (3.1)$$

$$dB/dt = k_8B_0 - k_1BX - k_3ABY, \quad (3.2)$$

$$dX/dt = k_1BX - 2k_2X^2 + 3k_3ABY - k_4X + k_6X_0, \quad (3.3)$$

$$dY/dt = 2k_2X^2 - k_3ABY - k_5Y, \quad (3.4)$$

where *A* denotes oxygen, *B* denotes NADH and *X* and *Y* represent free radical intermediates. The rate constant *k*₁ is proportional to the enzyme concentration. The expression *k*₇(*A*₀ − *A*) simulates the inlet of O₂ and the expression *k*₈*B*₀ (*B*₀ is constant) simulates the constant influx of NADH.

In the absence of perturbations this model will undergo a transition to chaos through a complicated series of bifurcations as the rate constant *k*₁ is increased (Fig. 2a) or through a series of period doubling bifurcations if *k*₁ is decreased. In addition the model has bistable periodic and chaotic attractors for certain values of *k*₁ (L. F. Olsen, un-

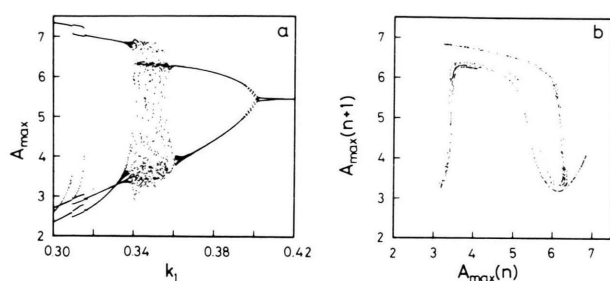


Fig. 2. Dynamics of the unperturbed model of the peroxidase-oxidase reaction ((3.1)–(3.4)). a) Bifurcation diagram obtained by varying k_1 (proportional to the enzyme concentration in the experimental system) and plotting the amplitudes of A following the dying out of transients. b) Next-amplitude plot of the oscillations of A for $k_1 = 0.35$. Other rate constants were $k_2 = 2.5 \cdot 10^2$, $k_3 = 3.5 \cdot 10^{-2}$, $k_4 = 20$, $k_5 = 5.35$, $k_6 X_0 = 10^{-5}$, $k_7 = 0.1$, $A_0 = 8.0$, $k_8 B_0 = 0.825$. The numerical simulations were done using a Runge-Kutta-Merson integration procedure.

published results). In contrast to the narrow parameter intervals yielding chaotic solutions in other models of biochemical and chemical reactions [9–11], the interval of k_1 resulting in chaotic dynamics is relatively broad and comparable in size to the corresponding enzyme concentration range in the experimental system. Finally a next-amplitude plot of the numerical data (Fig. 2b) is in remarkably good agreement with the corresponding plots of experimental data [7].

4. Periodic Perturbations of the Model

As evident from Fig. 1 the fluctuations of inlets are very small. Nevertheless, we know from the investigations of other models of chemical reactions, e.g. models of the Belousov-Zhabotinskii reaction [12, 13], that such small perturbations may be grossly amplified by the dynamics of the system, resulting in non-periodic responses indistinguishable from chaotic dynamics. Consequently it was decided to probe the present system by adding periodic perturbations to the inlets of reactants A and B using perturbations with an amplitude and frequency as estimated from the experimental data. Figure 3 shows the effects of periodically perturbing the inlet of B by modifying the expression $k_8 B_0$ in (3.2) to

$$k_8 B_0 + C \sin(2\pi t), \quad (4)$$

where C is a constant and t is the time. The perturbations are indicated in the left panel of Figure 3. We observe from the bifurcation diagrams to the right that the perturbations in this case result in minor expansions of the chaotic regime and small distortions of periodic solutions, in particular around the bifurcation points. The distortions are so small that they would be very difficult to detect experimentally.

The perturbations of the inlet of A were introduced by perturbing A_0 (corresponding to the O_2 content of the gas phase) in (3.1) by the expression

$$A_0(1 + D \sin(2\pi t/100)), \quad (5)$$

where D is a constant. Figure 4 shows the effect on the dynamics of increasing D . The panel on the left is included to illustrate the magnitude of the perturbation (compare Figure 1b). Again we observe that perturbations comparable to or larger than those estimated from the experimental results do not alter the dynamics significantly.

Finally the combined effects of perturbations of supplies of both reactants A and B were recorded as illustrated by the bifurcation diagram and the next-amplitude plot in Figure 5. We conclude from these results that small periodic perturbations with amplitudes and frequencies as in the experimental system do not change the dynamics of the reaction to any large extent.

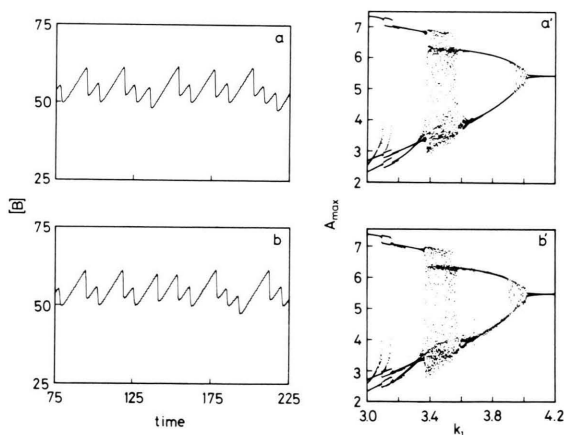


Fig. 3. Effects of periodic perturbations of the inlet of B on the dynamics of the model. The left panel shows the oscillations of B in the presence of increasing amplitudes of perturbations. The right panel shows the resulting bifurcation diagrams. B was perturbed using (4). a and a', $C = 0.5$; b and b', $C = 1.0$. Other parameters as in Figure 2.

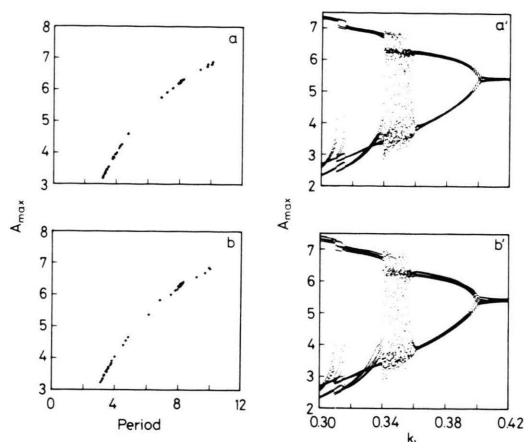


Fig. 4. Effects of periodic perturbations of the inlet of A on the dynamics of the model. The left panel shows plots of the amplitudes of oscillations of A against the period of oscillation. The right panel shows the resulting bifurcation diagrams. A was perturbed using (5). a and a' , $D = 0.005$; b and b' , $D = 0.01$. Other parameters as in Figure 2.

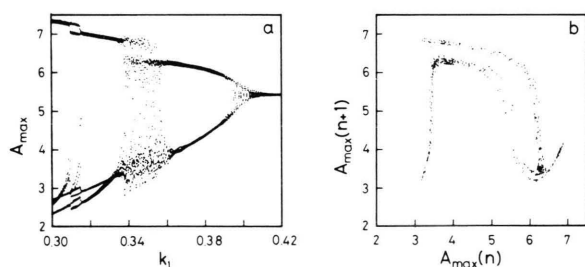


Fig. 5. Effects of periodic perturbations of inlets of both A and B on the dynamics of the model. a) Bifurcation diagram; b) next-amplitude plot for $k_1 = 0.35$. $C = 0.5$ and $D = 0.005$. Other parameters in Figure 2.

5. Random Perturbations of the Model

As indicated earlier it cannot be excluded that the inlet of NADH in the experimental system is also perturbed by random fluctuations. Consequently the effects of such perturbations of the inlet of B in the model were investigated. Fluctuations were introduced by adding a random term to the numerical solution of B . At each integration step the value of B was modified by a term given by the expression

$$Fh(0.5 - g(m)), \quad (6)$$

where F is a constant, h is the step length of integration, g is a function generating random numbers between 0 and 1 using an integer as the input

variable and m is the integer part of the time. Figure 6 shows the changes in dynamics of the model following increases in F . The traces in the panel to the left are included to indicate the noise level of B . We observe that increases in the noise are accompanied by a gradual loss of the fine structure of the bifurcation diagram.

An important question is then: What are the implications of random perturbations for the originally periodic and chaotic dynamics? This question is partially answered by comparing the next-amplitude plots of the perturbed periodic and chaotic solutions at different levels of noise as shown in Figure 7. We note that as the noise is increased the originally periodic motion gradually turns into random motion as shown by the formation of next-amplitude maps with decreasing order. In contrast the chaotic dynamics remains essentially chaotic and the overall structure of the next-amplitude map is preserved. We may conclude from these results that small random perturbations of the peroxidase-oxidase model may totally

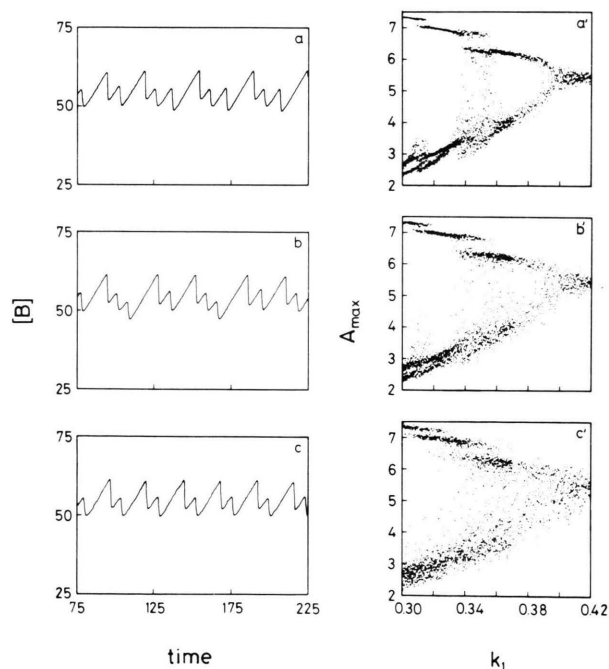


Fig. 6. Effects of random perturbations of the inlet of B on the dynamics of the model. The left panel shows the oscillations of B with the perturbations superimposed. The right panel shows the resulting bifurcation diagrams. Perturbations were made using (6). a and a' , $F = 0.5$; b and b' , $F = 1.0$; c and c' , $F = 2.0$. Other parameters as in Figure 2.

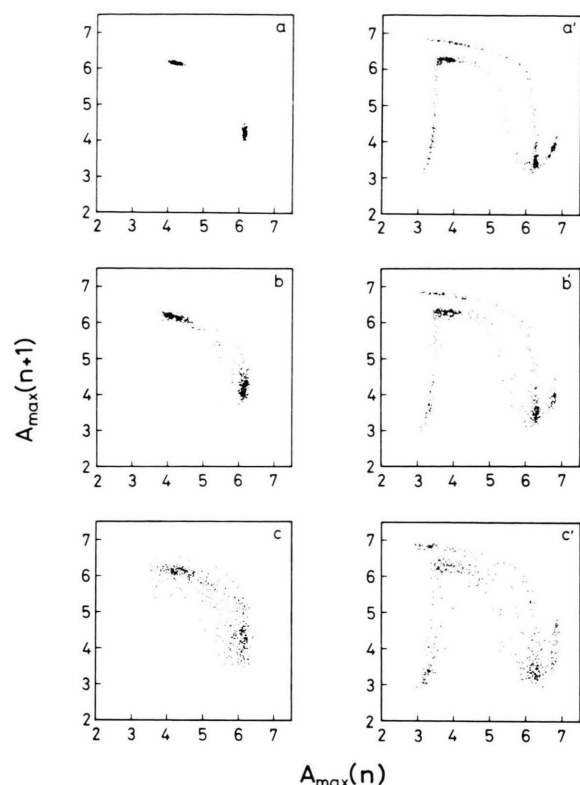


Fig. 7. Effects of random perturbations of the inlet of B on the next amplitude plots of a periodic oscillation (left panel) and a chaotic oscillation (right panel). Perturbations were made using (6). a and a' , $F = 0.5$; b and b' , $F = 1.0$; c and c' , $F = 2.0$. In the left panel $k_1 = 0.375$; in the right panel $k_1 = 0.35$. Other parameters as in Figure 2.

obscure the dynamics of simple periodic solutions whereas chaotic solutions seem to be a great deal less sensitive.

6. Discussion

The present study was performed to answer the following question: Can small periodic or stochastic perturbations induce a transition from periodic to chaotic behaviour in the peroxidase-oxidase reaction and hence obscure any chaotic motion resulting from the chemical dynamics itself? The answer to the above question may be split into two parts, one relating to the specific system and one relating to dynamical systems in general.

Let us consider the specific system first. The peroxidase-oxidase reaction is only one out of several chemical and biochemical systems for which chaos has been reported. In some of these systems, e.g. in the glycolytic system [14, 15], chaos can only be induced when the inlets of reactants are perturbed periodically. It is well known that adding a periodic perturbation to a periodically oscillating system will induce chaos in that system [16]. In other systems, e.g. in the Belousov-Zhabotinskii reaction, chaotic dynamics is believed to be an intrinsic property by some researchers [10, 17, 18], whereas others have argued that a large part of such dynamics observed experimentally may result from small random or periodic fluctuations of the inlets of reactants that are amplified by the chemical dynamics of the system [12, 13, 19]. These arguments may also apply to the present system. We observe here that periodic perturbations comparable in size or larger than the perturbations in the experimental system do not cause a transition from periodic to chaotic behaviour. Random perturbations on the other hand result in a transition from periodic motion to stochastic motion. However, this latter motion is clearly distinguishable from the chaotic motion caused by the chemical dynamics in that very little or no structure is present in the next-amplitude plot. Consequently small perturbations of the inlets of reactants do not obscure the chaotic dynamics in the present system.

More generally the present work extends previous studies of the effects of noise on simple chaotic systems. Schaffer and Kot [20] investigated the effects of random perturbations on periodic and chaotic dynamics in one-dimensional difference equations. They concluded that simple periodic motion may be totally obscured by the presence of low levels of noise. However, as the motion generated by the unperturbed map became more complex and finally chaotic more and more of the original dynamics was retained following addition of noise. Schaffer, Ellner, and Kot [21] extended these studies to systems of differential equations with essentially the same result namely that chaotic motion as opposed to simple periodic motion is relatively robust to external noise. These observations are confirmed by the present results obtained with the peroxidase-oxidase reaction, the chaotic behaviour of which is more complex than those previously reported for biochemical and chemical systems.

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