Studies of the Chaotic Behaviour in the Peroxidase-Oxidase Reaction

Lars F. Olsen
Institute of Biochemistry, Odense University, Denmark

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This note presents experimental results with an enzyme-catalysed reaction with a continuous supply of the substrates in which we observe a transition from periodic behaviour to apparently nonperiodic behaviour by decreasing the enzyme concentration. A model of the reaction is also described. This model, based only on elementary reaction steps, can simulate the periodic behaviour observed experimentally. By changing only one rate constant the behaviour changes from periodic to quasiperiodic behaviour. The work of earlier investigators suggests that such quasiperiodic behaviour (toroidal oscillations) is a step towards chaotic behaviour.

Introduction

A chemical reaction in a homogeneous solution may exhibit a variety of types of dynamic behaviour such as bistability, oscillations or chaos. The possibility of the latter phenomenon in chemical systems was suggested only a few years ago by Rössler, who examined a few abstract reaction schemes involving at least 3 chemical species [1]. Since then some experimental observations have been made which may be interpreted as chaotic behaviour in chemical reaction systems [2–6]. A common feature of these systems is that they all involve a continuous supply of the reactants. Some of these experiments have been criticized by Showalter et al. [7] who suggested that the observed nonperiodic behaviour originates from uncontrolled fluctuations in the experimental system. At the same time Showalter et al. [7] expressed serious doubts about the possibility of finding truly chaotic behaviour in a chemical reaction scheme involving only elementary processes. This note describes an experimental biochemical system in which the transition from periodic behaviour to apparently nonperiodic behaviour occurs, and a model which can simulate the periodic behaviour and which also contains the conditions for the emergence of chaos

The reaction in question is the oxidation of reduced nicotinamide adenine dinucleotide (NADH) by molecular oxygen catalysed by peroxidase. This reaction — named the peroxidase-oxidase reaction — is capable of demonstrating damped oscillations in a system open to oxygen [8, 9] and sustained oscillations [10, 11] as well as complex dynamic behaviour [2, 12] when both substrates are supplied continuously.

Methods and Materials

Experiments were performed in a 4.5 ml hexagonal glass cuvette fitted with a stirrer for efficient mixing. Oxygen was supplied to the reaction mixture from the gas phase above the liquid, containing a mixture of O_2 and N_2. If the oxygen partial pressure in the gas is constant, the rate of diffusion of oxygen into the liquid follows the simple rate law [13]

$$d[O_2]/dt = k([O_2]_{eq} - [O_2]),$$

where $[O_2]$ denotes the oxygen concentration in the liquid, $[O_2]_{eq}$ is the oxygen concentration in the liquid when the gas and the liquid are in equilibrium with respect to oxygen, and k is a constant. A solution of NADH was infused into the reaction mixture through a capillary, whose tip was below the surface of the liquid, using a high precision infusion pump (Harvard Apparatus Co., model 971).

The system was mounted in a dual wavelength spectrophotometer (Hitachi-Perkin Elmer 356). NADH was measured spectrophotometrically at 380 nm minus 400 nm and oxygen was measured with a Clark-type electrode (Radiometer, Copenhagen). A detailed description of the system has appeared previously [11, 12].

Horseradish perioxidase (RZ 1.0) and NADH were obtained from Boehringer. Methylene blue and 2,4-dichlorophenol were purchased from Merck.

Computer simulations were performed on a minicomputer with peripherals using a standard Runge-Kutta-Merson integration procedure.

Results and Discussion

The waveform of the oscillations in the peroxidase-oxidase reaction with continuous supply of NADH and O_2 are very much dependent on the concentra-

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Reprint requests to Dr. L. F. Olsen, Institute of Biochemistry, Odense University, Campusvej 55, DK-5230 Odense M, Denmark.

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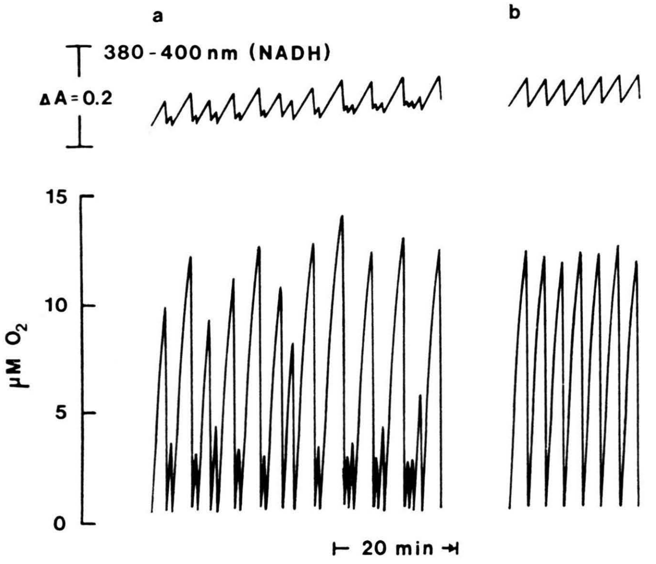

Fig. 1. Nonperiodic and periodic oscillations in the peroxidase-oxidase reaction. Experimental conditions: a) 0.7 μM peroxidase, 0.1 μM methylene blue and 11 μM 2,4-dichlorophenol in 0.1 M acetate buffer, pH 5.1. O_2 content in the gas phase was 1.65% by volume. 0.25 M NADH was infused at a rate of 12.5 μl/h. Temperature 28 °C. b) as in a) except that the enzyme concentration was increased to 1.2 μM.

tion of the enzyme as illustrated in Figure 1. It has previously been shown [2] that the oscillations shown in Fig. 1a may be of the chaotic type since plots of a given amplitude of the oscillation against the preceding amplitude (next amplitude map) defines a single valued bell-shaped curve satisfying Li and Yorke's [14] conditions for chaos.

The peroxidase-oxidase reaction can be simulated by a class of models involving linear and quadratic branching steps and linear termination [11, 12] as

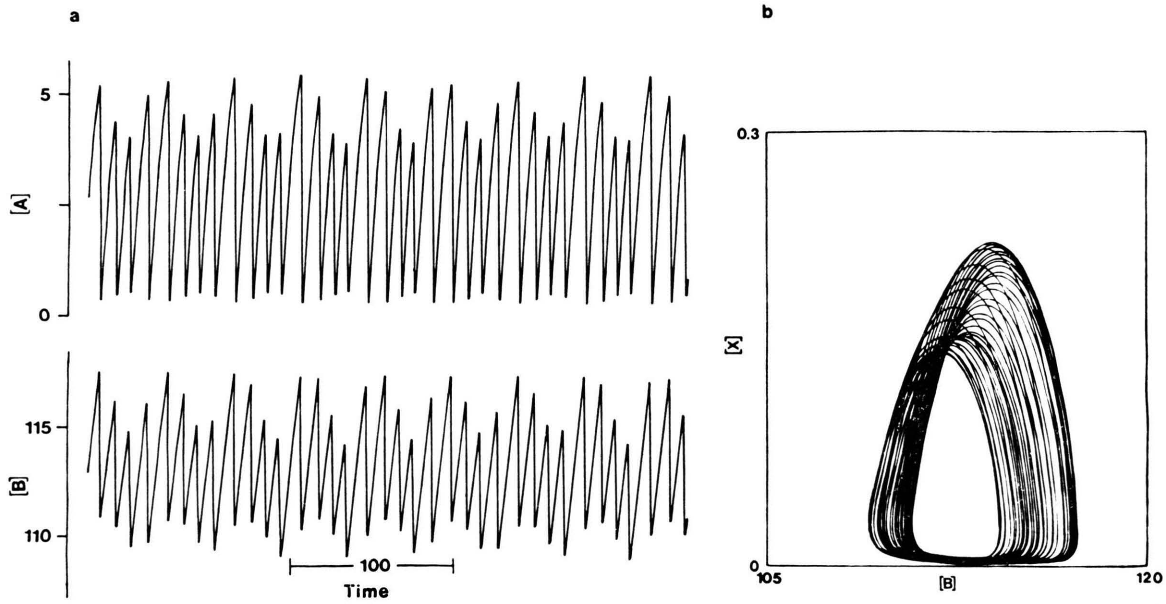

Fig. 2. a) Nonperiodic behaviour in the model described in the text. Rate constants: $k_1 = 0.165$, $k_2 = 3.75 \times 10^2$, $k_3 = 3.5 \times 10^{-2}$, $k_4 = 20.0$, $k_5 = 5.35$, $k_6 = 10^{-3}$, $k_7 = k_{-7} = 0.1$, $k_8[B_0] = 0.825$, $[A_0] = 8.0$. Initial conditions: $[A] = 8.0$, $[B] = 100.0$, $[X] = [Y] = 0.0$. The traces shown begin at approximately 400 time units after the simulation was started. b) Plot of [X] against [B] for the oscillations shown in Figure 2a.

shown below

$$\begin{array}{rr} \mathrm{B} + \mathrm{X} \xrightarrow{k_1} 2\,\mathrm{X}, & 2\,\mathrm{X} \xrightarrow{k_2} 2\,\mathrm{Y}, \\ \mathrm{A} + \mathrm{B} + \mathrm{Y} \xrightarrow{k_3} 2\,\mathrm{X}, & \mathrm{X} \xrightarrow{k_4} \mathrm{P}, \\ \mathrm{Y} \xrightarrow{k_5} \mathrm{Q}, & \mathrm{X}_0 \xrightarrow{k_6} \mathrm{X}, \\ \mathrm{A}_0 \underset{k_{-7}}{\overset{k_7}{\rightleftarrows}} \mathrm{A}, & \mathrm{B}_0 \xrightarrow{k_8} \mathrm{B}. \end{array}$$

Here A denotes oxygen, B denotes NADH and X and Y are intermediate free radicals. The sixth reaction accounts for the spontaneous formation of free radicals in the reaction mixture. This model is a modification of a model proposed by Degn to explain the oscillations in the Bray reaction [15, 16]. The model can simulate the periodic behaviour observed in the experimental system. By changing only one parameter (k_1) apparently nonperiodic oscillations are observed as shown in Figure 2a. These oscillations are not of the same type as those shown in Figure 1a. The next amplitude map is a closed curve and therefore cannot be used for diagnosing chaos. The flow appears to be confined to a distorted torus (or its 4-dimensional analog) a illustrated in Figure 2b. These oscillations are therefore quasiperiodic and not truly chaotic. However, Rössler has recently presented some simple differential equations in which the transition toroidal behaviour → chaotic behaviour occurs [17, 18]. Hence a more detailed analysis may reveal that this transition also occurs in the model described here because as shown by Rössler toroidal oscillation is a possible (though not necessary) step towards chaos. Such an analysis is now in progress.

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