AN ENZYME REACTION WITH A STRANGE ATTRACTOR

Lars F. OLSEN
Institute of Biochemistry, Odense University, Campusvej 55, DK-5230 Odense M, Denmark

Received 13 October 1982
Revised manuscript received 2 February 1983

A simple model of the oscillating peroxidase–oxidase reaction is presented. The model is capable of reproducing all previously observed dynamic patterns exhibited by the experimental system including its chaotic behaviour.

1. Introduction. It is only a few years ago that Rössler proposed that chaos may be found in a chemical system open to its surroundings [1]. Shortly afterwards experimental evidence for chaos was reported for an oscillating enzyme reaction: the peroxidase–oxidase reaction [2]. Since then numerous examples of chaotic behaviour have been found in both abstract and real chemical systems [3].

In the peroxidase–oxidase reaction, which is the subject of the present work, an organic substance, usually reduced nicotinamide adenine dinucleotide (NADH), is oxidised with molecular oxygen as the electron acceptor:

$$O_2 + 2NADH + 2H^+ \rightarrow 2H_2O + 2NAD^+ .$$

The reaction is catalysed by the enzyme peroxidase (EC 1.11.1.7). Depending on the amount of enzyme present in the reaction mixture, the system will exhibit both periodic and nonperiodic oscillations when both substrates, NADH and O_2, are continuously supplied [2,4]. An example is shown in fig. 1. Following an earlier suggestion by Rössler [1], the apparently nonperiodic oscillations in fig. 1b were analysed by plotting the amplitude of each excursion against the amplitude of the previous excursion as illustrated in fig. 2. Originally this plot was thought to define a single-valued bell-shaped function [2] for which evidence for chaotic behaviour could be established by help of Li and Yorke's theorem [5].

Using a model proposed for the oscillating Bray re-

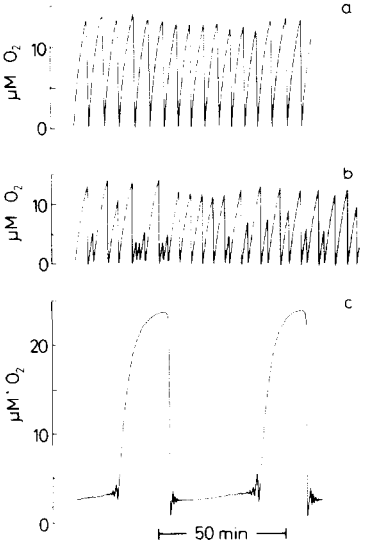

Fig. 1. Effect of the enzyme concentration on the oscillations in O_2 concentration in the peroxidase–oxidase reaction. The experimental system consisted of a 5 ml stirred aqueous solution containing horseradish peroxidase, 10 μM 2,4-dichlorophenol, 0.2 μM methylene blue and 0.1 M Na-acetate, *pH* 5.1. O_2 entered the reaction mixture from a N_2/O_2 gas phase above the liquid containing 1.9 vol% O_2. A 0.2 M solution of NADH was pumped into the reaction mixture at a constant rate of 11 μl per hour. The oxygen concentration in the liquid was measured with a Clark electrode. The temperature was 28°C. The concentration of peroxidase was (a) 0.9 μM, (b) 0.55 μM, (c) 0.4 μM.

0 031-9163/83/0000–0000/$ 03.00

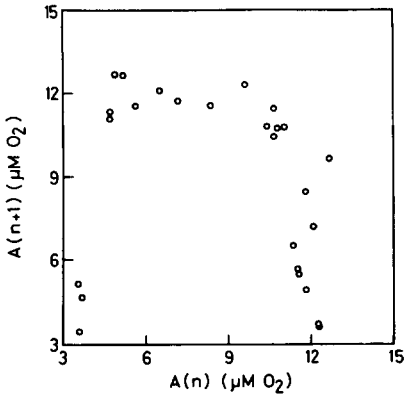

Fig. 2. Next-amplitude plot of the oscillations from fig. 1b.

action [6] it has been possible to construct a model of the peroxidase–oxidase reaction which is capable of reproducing several of the dynamic features of the experimental system [7]. However, one of the shortcomings of the original model [7] is that it can only reproduce the periodic oscillations observed in the experimental system. Here I present a slightly modified version which, in addition to periodic attractors, apparently also contains at least one chaotic attractor. In addition to giving remarkably good reproductions of all previously observed oscillatory patterns [2,4,7] this model allows for a reinterpretation of the chaotic behaviour in the experimental system [2].

2. Description of the model. The peroxidase–oxidase reaction may involve as many as 20 individual reaction steps, some of which are not known in detail [7,8]. Furthermore data on individual rate constants are scarce. However, some of the basic features of the reaction are now well understood. For instance it is known that the reaction is a branched chain reaction with autocatalysis and that the enzyme participates in at least one branching reaction [7,8]. The present model which is a modification of a previously described model [7] incorporates these features by inclusion of two linear branching reactions, one quadratic branching reaction and two linear termination steps. The model is presented below:

$$B + X \xrightarrow{k_1} 2X\,, \tag{1}$$

$$2X \xrightarrow{k_2} 2Y\,, \tag{2}$$

$$A + B + Y \xrightarrow{k_3} 3X\,, \tag{3}$$

$$X \xrightarrow{k_4} P\,, \tag{4}$$

$$Y \xrightarrow{k_5} Q\,, \tag{5}$$

$$X_0 \xrightarrow{k_6} X\,, \tag{6}$$

$$A_0 \underset{k_7}{\overset{k_7}{\rightleftharpoons}} A\,, \tag{7}$$

$$B_0 \xrightarrow{k_8} B\,. \tag{8}$$

Here A corresponds to O_2, B corresponds to NADH and X and Y represent intermediate free radicals. Reaction (1) is considered to be the branching reaction involving the enzyme and k_1 therefore depends linearly on the enzyme concentration. Reaction (6) accounts for the spontaneous (slow) formation of free radicals in the absence of which the reaction would never start. Reactions (7) and (8) simulate the inputs of O_2 and NADH from their respective sources (gas phase with a constant partial pressure of O_2 and a concentrated NADH solution infused into the reaction mixture at a constant rate). The corresponding differential equations to be solved are

$$\dot{A} = k_7(A_0 - A) - k_3ABY\,, \tag{9}$$

$$\dot{B} = k_8B_0 - k_1BX - k_3ABY\,, \tag{10}$$

$$\dot{X} = k_1BX - 2k_2X^2 + 3k_3ABY - k_4X + k_6X_0\,, \tag{11}$$

$$\dot{Y} = 2k_2X^2 - k_3ABY - k_5Y\,. \tag{12}$$

The equations were integrated numerically on a UNIVAC 1100 computer using a standard Runge–Kutta–Merson procedure.

3. Results and discussion. Fig. 3 shows the oscillations of A for three different values of k_1. The oscillations in fig. 3b are apparently nonperiodic. The dynamics of the model was investigated for the interval $0.3 \leqslant k_1 \leqslant 0.41$ by changing the value of k_1 in steps of 0.001. The result of this investigation is shown in fig. 4. Periodic regimes are denoted P_i, where i is the number of maxima per period. Except for the transition $P_2 \rightarrow P_3$, transitions between successive periodic regimes are rather sharp, i.e. there was no indication

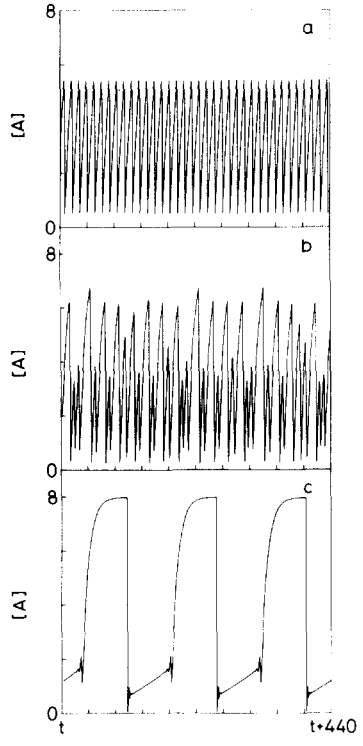

Fig. 3. Oscillations of A for three different values of k_1: (a) $k_1 = 0.41$, (b) $k_1 = 0.35$, (c) $k_1 = 0.16$. Other parameters are: $k_2 = 2.5 \times 10^2$, $k_3 = 3.5 \times 10^{-2}$, $k_4 = 20$, $k_5 = 5.35$, $k_6 X_0 = 10^{-5}$, $k_7 = 0.1$, $A_0 = 8$, $k_8 B_0 = 0.825$. Units of time and concentration are dimensionless.

of nonperiodic regimes bounded by periodic regimes other than that separating P_2 and P_3. The two shaded regimes represent oscillations with rapidly increasing periods (period doublings) as the nonperiodic regime is approached.

A stability test was performed for the numerical solutions of eqs. (9)–(12) for the various regimes shown in fig. 4. Following small perturbations, oscillations in the periodic regimes rapidly converged to

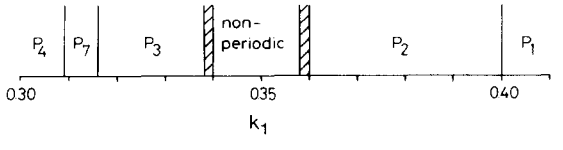

Fig. 4. Sequence of oscillatory patterns obtained by varying k_1 and keeping the other parameters constant (values as in fig. 3). Periodic regimes are denoted P_i, where i is the number of excursions per period.

the unperturbed oscillations, whereas oscillations in the nonperiodic regime rapidly diverged from the unperturbed oscillations. In the latter case a completely different dynamic pattern was obtained within 20 excursions for even the smallest perturbations.

A one-dimensional map was constructed for the oscillations shown in fig. 3b by plotting successive amplitudes against the preceding amplitude (fig. 5a). The map consists of bundles of "curves" between which the system jumps in an apparently erratic manner. The map has a structure reminiscent of the structure of Hénon's map [9]. The map in fig. 5a further resembles Hénon's map in having a transversal substructure as illustrated by fig. 5b, which is a magnification of the square region indicated in fig. 5a. Each of the "curves" in fig. 5a turns out to consist of a bundle of subcomponents. An enlargement of a small square region of the map in fig. 5b revealed that this multiplication of curves apparently continues. Further magnifications were not made owing to the large number of maxima ($>10^5$) that would have to be computed. Inspection of the transversal structures of successive magnifications of the map in fig. 5a revealed that the new structure obtained after a magnification appears to be the mirror image of the original structure before the magnification. This suggests that the attractor represented by the map in fig. 5b has a "Cantor-set like" structure.

The possibility that the oscillations shown in fig. 3b represent a transient state preceding an oscillating state with a finite period cannot at present be excluded. However, the numerical results presented above suggest that these oscillations are chaotic. The evidence for chaotic behaviour is briefly summarized:

(i) The dynamic pattern does not repeat itself within several tens of thousands of excursions.

(ii) The pattern is highly sensitive to initial conditions and diverges rapidly following small changes in these. This is a characteristic feature of chaotic systems [10,11].

(iii) The transition from periodic to nonperiodic behaviour occurs through a series of period doublings [12].

For a minimal model, the present reaction scheme gives remarkably good reproductions of the experimentally observed dynamic patterns (compare figs. 1 and 3), and the changes in these resulting from changes in a single rate constant parallel the experi-

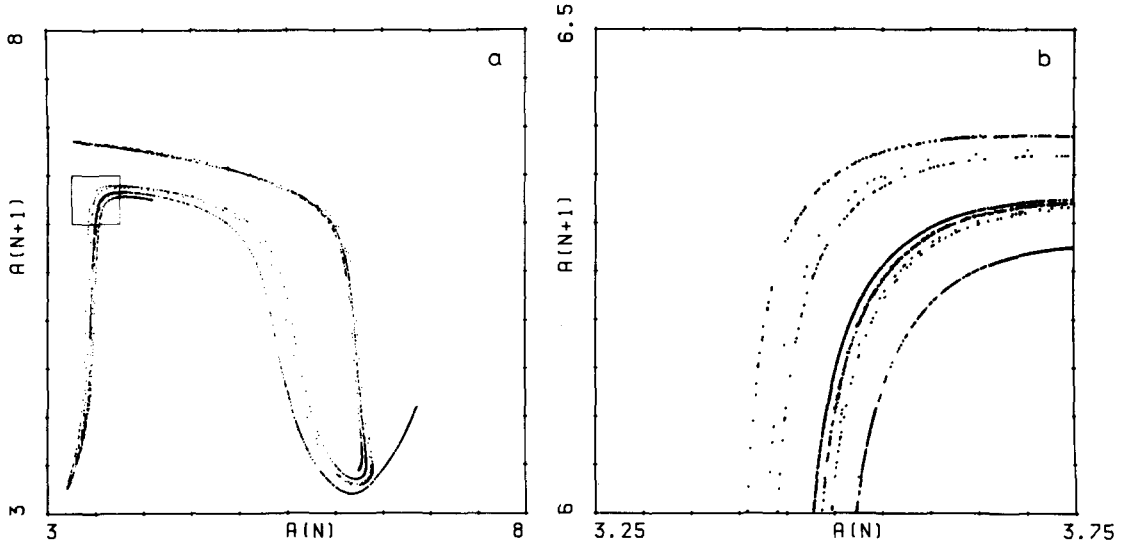

Fig. 5. Next-amplitude plots of the oscillations shown in fig. 3b. (a) 3000 maxima have been computed. The first of these maxima is preceded by 1000 maxima that were discarded. (b) Magnification of the square region shown in (a), 18000 maxima have been computed.

mentally observed changes induced by varying the enzyme concentration. Furthermore, the next-amplitude plot presented in fig. 5a allows for a reinterpretation of the corresponding map obtained for the experimental system (fig. 2). Inspection of the data points in fig. 2 reveals that these may well fit onto a map like the one shown in fig. 5a. The dynamical behaviour of the peroxidase–oxidase reaction may thus be more complex than the behaviour previously reported for the Belousov–Zhabotinskii reaction [13]. To summarize it has now been possible to link up experimental observations of complex dynamic behaviour in the peroxidase–oxidase reaction with a realistic reaction scheme. A similar link between model and experiment has recently been demonstrated for the Belousov–Zhabotinskii reaction [14].

Acknowledgements. The author wishes to thank Dr. H. Degn for many helpful suggestions and for criticism of the manuscript. Professor P.E. Phillipson, University of Colorado, is acknowledged for helpful discussions on topics related to this work. This research was supported by the Danish Natural Science Research Council.

[1] O.E. Rössler, Z. Naturforsch. 31a (1976) 259; Bull. Math. Biol. 39 (1977) 276.
[2] L.F. Olsen and H. Degn, Nature 267 (1977) 177.
[3] K. Tomita, Phys. Rep. 86 (1982) 113;
V. Hlavacek and P. Van Rompay, Chem. Eng. Sci. 36 (1981) 1587; see also Ann. N.Y. Acad. Sci. 316 (1979); 357 (1981).
[4] S. Nakamura, K. Yokota and I. Yamazaki, Nature 222 (1969) 794;
I. Yamazaki and K. Yokota, Mol. Cell. Biochem. 2 (1973) 39.
[5] T.-Y. Li and J.A. Yorke, Am. Math. Month. 82 (1975) 985.
[6] P. Lindblad and H. Degn, Acta Chem. Scand. 21 (1967) 791;
H. Degn, Acta Chem. Scand. 21 (1967) 1057.
[7] L.F. Olsen and H. Degn, Biochim. Biophys. Acta 523 (1978) 321;
L.F. Olsen, Z. Naturforsch. 34a (1979) 1544.
[8] H. Degn, Biochim. Biophys. Acta 180 (1969) 271;
H. Degn and D. Mayer, Biochim. Biophys. Acta 180 (1969) 291.
[9] M. Hénon, Commun. Math. Phys. 50 (1976) 69.
[10] H. Degn, Phys. Rev. A26 (1982) 711.
[11] R. Shaw, Z. Naturforsch. 36a (1981) 80.
[12] M.J. Feigenbaum, J. Stat. Phys. 19 (1978) 25; 21 (1979) 669.
[13] J.L. Hudson and J.C. Mankin, J. Chem. Phys. 74 (1981) 6171;
R.H. Simoyi, A. Wolf and H.L. Swinney, Phys. Rev. Lett. 49 (1982) 245.
[14] J.S. Turner, J.-C. Roux, W.D. McCormick and H.L. Swinney, Phys. Lett. 85A (1981) 9.