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Oscillations in peroxidase-catalyzed reactions and their potential function in vivo

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Abstract

The peroxidase-oxidase reaction has become a model system for the study of oscillations and complex dynamics in biochemical systems. In the present paper we give an overview of previous experimental and theoretical studies of the peroxidase-oxidase reaction. Recent in vitro experiments have raised the question whether the reaction also exhibits oscillations and complex dynamics in vivo. To investigate this possibility further we have undertaken new experimental studies of the reaction, using horseradish extracts and phenols which are widely distributed in plants. The results are discussed in light of the occurrence and a possible functional role of oscillations and complex dynamics of the peroxidase-oxidase reaction in vivo.

Keywords: Peroxidase-oxidase; Oscillations; Homoclinic chaos; Horseradish; Phenols; Vanillin

1. Introduction

Oscillatory dynamics are considered to be of great importance in biochemical and biophysical reaction systems [1]. Over the last 20 years numerous examples of oscillating biochemical reactions have been reported. Nevertheless, in spite of intense studies of such oscillations, we still lack a general understanding of why cellular processes seem to prefer an oscillatory state over a steady state. On the one hand, we appear to understand the function of the non-linear properties of ion channels in the plasma membrane of a heart cell as a prerequisite for the rhythmic contraction of the heart muscle [2], and the function of an endogenous circadian rhythm as a mechanism of adaptation to changes in environment [3]. On the other hand, there are many oscillating biochemical processes whose function we do not understand. Examples are the pulsatile secretion of insulin by pancreatic β-cells in laboratory animals as well as in humans [4,5] and the hormone-receptor induced oscillations in the concentration of intracellular calcium in a large number of eucaryotic cells [6,7].

The increasing evidence for the importance of biochemical and biophysical oscillatory processes has brought new life to the study of simple biochemical oscillating reactions. The simplest conceivable biochemical oscillators are single enzyme systems which oscillate in homogeneous phase. Simple biochemical oscillators include a biomimetic cytochrome P450 model system [8], a catalase/ascorbate/oxygen system [9], a peroxidase/H_2O_2/quinone electrochemi-

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cal system [10–12], and the peroxidase-oxidase (PO) reaction [13,14]. Among these the PO reaction, which involves a single enzyme, two substrates and two modifiers of activity, is by far the best understood. The overall stoichiometry of the PO reaction is the following:

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

where NADH and NAD^+ represent the reduced and oxidized forms of nicotinamide adenine dinucleotide, respectively. Strictly speaking, the name peroxidase-oxidase reaction is not limited to the oxidation of NADH by O_2, but also includes the oxidation of three other substrates (indoleacetic acid, dihydroxyfumaric acid and triose reductone) by molecular oxygen [15]. However, up to now the reaction where NADH is the hydrogen donor is the most studied, and hence the term PO reaction is often used synonymously with this particular reaction.

Observation of sustained oscillations in the PO reaction requires that both NADH and O_2 are continuously supplied to the reaction mixture. Furthermore, in the absence of any modifier the reaction only shows damped oscillations [16,17]. However, if provided with methylene blue (MB) and a suitable aromatic compound, the reaction exhibits sustained periodic and complex periodic oscillations as well as chaotic dynamics [18–20]. In most of the studies 2,4-dichlorophenol (DCP) was used to induce oscillations and complex dynamics. However recent studies have shown that other aromatic compounds may have the same or a similar effect to DCP [20]. Those aromatic compounds which will induce oscillations and complex dynamics are all substrates in a related peroxidase-catalyzed reaction where hydrogen peroxide replaces oxygen as the oxidizing agent:

$$H_2O_2 + 2YH \rightarrow 2H_2O + 2Y^{\bullet} \quad (2)$$

We would like to emphasize that many of these aromatic compounds occur in intact organisms.

Most studies of oscillations have been made using peroxidase from horseradish roots, but peroxidases from other organisms, such as fungi and mammals, also catalyze an oscillating reaction [21]. Thus, from being just another model system for the study of oscillations in biochemical reaction systems, the PO reaction is beginning to grow physiological flesh.

In the present article we have investigated the PO reaction in the presence of a number of natural phenols which are widely distributed in plants [22]. Furthermore, we have studied the dynamical response of extracts of horseradish roots to continuous supplies of NADH. The purpose of these studies was to shed new light on the occurrence of oscillations in vivo.

The rest of this paper is organized as follows: in Section 2 we present Materials and Methods. In Section 3 we give a brief overview of previous important results with respect to oscillations and complex dynamics in the PO reaction. In Section 4 we present new results obtained by replacing DCP by naturally occurring phenolic substrates and by using extracts of horseradish roots instead of the purified enzyme. In Section 5 we discuss the possible function of oscillations in the PO reaction in the intact plant.

2. Materials and methods

NADH (grade II) was purchased from Boehringer Mannheim and dissolved to a 0.1 M stock solution in distilled water. Horseradish peroxidase was purchased from Boehringer Mannheim (RZ 3.2) or from Sigma (RZ 3.0). Methylene blue was obtained from Merck; 2,4-dichlorophenol and vanillic acid were obtained from Aldrich; 4-hydroxybenzoic acid and ferulic acid were from Sigma; 2-hydroxycinnamic acid was supplied by Fluka. All other aromatic compounds used in this study (i.e. salicylic acid, guaiacol, vanillin and 4-hydroxybenzaldehyde) were kindly made available by the Department of Chemistry, Odense University. The aromatic compounds were added to the reaction mixture from a concentrated stock solution (usually 10 mM) in 99.9% ethanol.

Extracts of horseradish roots were made using a modified procedure of the one described by Elstner and Heupel [23]: 30–65 g horseradish root was sliced and homogenized in ice-cold distilled water (3 ml of H_2O per g tissue) using an OBH (Copenhagen) rod-blender. The homogenate was filtered through cheesecloth and centrifuged at $1000 \times g$ for 20 min at 8°C in a Sorvall RC 5C centrifuge. The pellet was resuspended in 100 ml ice-cold distilled water and recentrifuged. Resuspension and recentrifugation were repeated twice and the final pellet was resuspended in ice-cold distilled water corresponding to 7 ml of

water per 30 g of horseradish root. The extract was either used directly without further treatment or stored at −20°C for later use.

Measurements of dynamics were performed at 28°C in a 18 × 20 × 37 mm quartz cuvette from Hellma placed in a Shimadzu 1201 single beam spectrophotometer. For experiments with purified peroxidase we used a 7 ml stirred sample, containing 0.1 M Na-acetate buffer (pH 5.1) or 0.1 M Na-phosphate buffer (pH 6.3), 1.3–1.6 μM peroxidase, 0.05–0.1 μM MB, and phenolic compound in different concentrations. A 0.1 M solution of NADH was infused at a flowrate of 19–40 μl/h through a capillary tube from a 250 μl Hamilton syringe mounted in a high precision syringe pump (Harvard Apparatus, model 22). The gas phase above the liquid was controlled by blowing a 1.05% (v/v) O_2/N_2 mixture over the surface of the

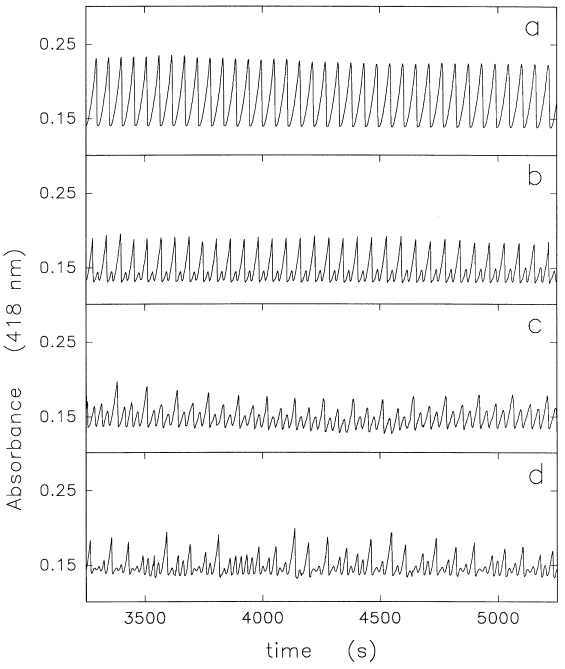

Fig. 1. Period-doubling route to chaos at pH 5.2. Time series of the absorbance changes at 418 nm where oxyferrous peroxidase (compound III) has its absorption maximum. The dynamics in (a) to (d) result from an increase in the mean NADH concentration in the solution, which again corresponds to an increase in the NADH infusion rate. The response is as follows: (a) p_1 oscillation; (b) p_2 oscillation; (c) p_4 oscillation; (d) chaos. Experimental conditions are 1.6 μM peroxidase, 0.1 μM MB, and 25 μM 2,4-dichlorophenol. The mean NADH concentrations are (a) 65 μM, (b) 73 μM, (c) 75 μM, and (d) 76 μM. Other conditions as listed in Section 2.

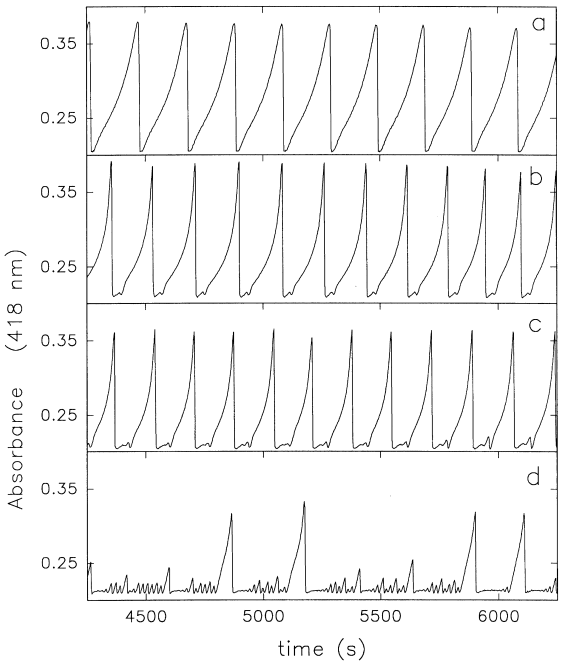

Fig. 2. Period-adding route to chaos at pH 6.3. Time series of the absorbance changes at 418 nm. The dynamics in (a) to (d) result from an increase in the mean NADH concentration in the solution. The response is as follows: (a) 1^0 oscillation; (b) 1^1 oscillation; (c) 1^2 oscillation; (d) chaos. Experimental conditions are 1.5 μM peroxidase, 0.1 μM MB, and 25 μM 2,4-dichlorophenol. The mean NADH concentrations are (a) 105 μM, (b) 113 μM, (c) 118 μM, and (d) 125 μM. Other conditions as listed in Section 2.

reaction mixture. The transfer rate of oxygen V_{O2} into the liquid is then given by:

$$V_{O_2} = K([O_2]_{eq} - [O_2]) \quad (3)$$

where $[O_2]_{eq}$ is the oxygen concentration in the liquid at equilibrium and K is the oxygen transfer constant. In the present setup the oxygen transfer constant was $(4.1 \pm 0.2) \times 10^{-3}$/s at a stirring speed of 1000 rev./min. Oxygen in the solution was measured with a Radiometer (Copenhagen) electrode mounted on the side of the cuvette. The concentration of NADH was measured by monitoring the absorbance at 360 nm. Data from the oxygen electrode and the spectrophotometer were recorded digitally at 1 s intervals and stored on a computer.

A few experiments with purified peroxidase were performed in a 21.7 × 21.7 × 42 mm quartz cuvette placed in a Zeiss Specord S10 diode array spectrophotometer. The experiments were performed in the

same way as described above, except that the volume of the liquid was 10 ml, the oxygen transfer constant was $(6.0 \pm 0.2) \times 10^{-3}$/s (stirring speed 1050 rev./min), and the NADH flowrate was varied between 50 and 70 μl/h. In this setup a full spectrum in the range of 350–600 nm (resolution 1 nm) and the O_2 concentration in the liquid was sampled at 2 s intervals and stored on a computer.

Experiments with the horseradish root extracts were performed in the setup mounted in the Shimadzu spectrophotometer. The extract was placed undiluted into the cuvette. MB was added to a final concentration of 0.2 μM and NADH was supplied at a flowrate of 19–21 μl/h. Due to the high turbidity of the cell extracts only the oxygen concentration in the extract was sampled.

3. Previous results of simple and complex dynamics in the PO reaction

The first reports of oscillatory dynamics in the PO reaction were almost contemporary with the first observations of oscillations in glycolysis [16,18,24,25]. Furthermore, the PO reaction was one of the first among chemical reactions to show bistability [26] and the first reaction to show chaotic dynamics [19]. In addition to the coexistence of two stable steady states [26], oscillation-steady state bistability, i.e. the coexistence of a stable steady state and a stable limit cycle, were found recently [27].

In the last 5–6 years we have acquired a considerable amount of knowledge about the PO reaction and its mechanism due to intensive experimental studies of the reaction's complex dynamics. For example, it has been shown that the transition from simple periodic oscillations to chaotic dynamics may take place through at least two different routes: (1) A period-doubling route which predominates at low pH (pH $<$ 5.4) [28,29] and (2) a period-adding route which predominates at pH $>$ 5.4 [29,30]. The latter culminates in chaotic behaviour which was shown to be associated with a homoclinic orbit [30]. Figs. 1 and 2 show examples of the transitions to chaos observed at pH 5.2 and pH 6.3, respectively. The change from one dynamic state to the other is induced by increasing the mean concentration of NADH in the reaction vessel. The dynamic states shown in Fig. 1 include a simple period-1 (p_1) oscillation (Fig. 1a) which changes to a period-2 (p_2) oscillation (Fig. 1b) to a period-4 (p_4) oscillation (Fig. 1c), etc. and finally to chaos (Fig. 1d) as the mean NADH concentration is increased. For the dynamics observed at pH 6.3 we use a different notation, namely the L^S notation, where L is the number of large amplitude oscillations and S is the number of small amplitude oscillations per period. The dynamics in Fig. 2 changes from a simple (1^0) periodic oscillation (Fig. 2a) to a state (1^1) where one large-amplitude oscillation alternates with one small-amplitude oscillation (Fig. 2b), to a state (1^2) where one large-amplitude oscillation alternates with two small amplitude oscillations (Fig. 2c), etc. and finally to chaos (Fig. 2d). However, the dynamics at pH 6.3 are more complicated than indicated by the discussion above. Each 1^S state is separated from a neighbouring state (1^{S+1}) by both period-doubled ($(1^S)^{2n}$), concatenated (1^S1^{S+1}) and chaotic states [29,30]. Other types of dynamics observed in the PO system include a chaotic domain squeezed in between two periodic domains of a Farey sequence [31,32] and quasiperiodic oscillations [33,34].

For a long time the oscillating PO reaction was considered as nothing more than an interesting reaction system exhibiting exotic types of dynamics. Until recently few researchers would have believed that the oscillations and complex dynamics could have any physiological meaning. One reason for this is that oscillations and complex dynamics in the PO reaction require the presence of both MB and DCP [18,28,35]. In the absence of these modifiers of enzyme activity the reaction only shows steady state kinetics or damped oscillations. Neither MB nor DCP are known to occur naturally, and hence they have no natural physiological function. However, recently it was demonstrated that other aromatic compounds, including both phenolic compounds and aromatic amines, may replace DCP in inducing oscillations and complex dynamics [20]. All aromatic compounds which do so are also substrates for the normal peroxidase cycle (eqn (2)). Structurally similar compounds which can be expected to bind to the enzyme's active site, but which lack the aromatic hydroxy- or amino group, did not have any effect on oscillations [20]. However, not all of the compounds which induce oscillations are also able to induce complex dynamics. Recent experiments described below may help to

bring to light the mechanism responsible for this difference in action. Furthermore, many different peroxidase enzymes, both from animals and from plants, are able to catalyze an oscillating reaction [21]. These experimental studies together with studies of models of the PO reaction [17,36,37] have shown that one condition for observing oscillations is that oxyferrous peroxidase, also known as compound III, is readily formed and decomposed. By contrast, compound III is usually not formed in peroxidase-catalyzed reactions where hydrogen peroxide is the oxidant (eqn (2)).

As stated in Section 1 dihydroxyfumaric acid and indole acetic acid may replace NADH as substrates in the PO reaction (Eq. (1)). It has long been known that damped oscillations can be observed with these substrates [38]. Recently complex oscillations have also been demonstrated with dihydroxyfumaric acid as the substrate [39]. It is to be noted that these oscillations were obtained in the absence of MB or any aromatic compound. On the other hand, sustained and complex oscillations have not yet been observed with indole acetic acid as a substrate.

By now many of the individual reaction steps of the PO reaction have been identified and their rate constants determined [14]. This has led to the proposal of detailed models of the reaction [17,36,37,40,41]. All these models are derived from a scheme originally proposed by Yokota and Yamazaki [42]. The models are capable of simulating most of the various types of dynamics observed experimentally, including the period-doubling [36,37,41,43] and the period-adding [30,43] routes to chaos as well as the transition between them [29]. One of these models, the so-called BFSO scheme [37], is shown in Fig. 3. It is still an open question which of the reaction steps are responsible for the oscillatory dynamics. However, it was shown previously that complex oscillations in this and related models require at least two parallel pathways leading to the formation of compound III [36,37,43]. In the absence of the pathway $Per^{3+} \rightarrow Per^{2+} \rightarrow Per^{6+}$ (Fig. 3) only periodic oscillations are observed [17,36]. It is worth noting that, as in most other models, the aromatic compound and MB are not incorporated in the scheme. Instead these compounds are included as rate constants of some of the individual reaction steps [17,37]. Recently, an approach has been made to disclose the role of the aromatic compound [44]. It was shown that the ability of phenolic compounds (PhOH) to induce oscillations and complex behaviour in the PO reaction may be correlated with the half-wave potentials $E_{1/2}$ for the formation of the phenoxyl radical:

$$PhOH \rightarrow PhO^{\bullet} + e^{-} + H^{+} \quad (4)$$

Phenolic compounds with half-wave potentials less than 785 mV (vs. normal hydrogen electrode, NHE) or higher than approximately 1000 mV (vs. NHE) did not induce sustained oscillations. Phenolic compounds with half-wave potentials in the range 785 mV $< E_{1/2} <$ 1000 mV induced both simple and complex periodic oscillations as well as chaos. The most complex behaviours were observed with phenolic compounds with a half-wave potential in the range 890 mV $< E_{1/2} <$ 960 mV [44]. This observation will undoubtedly be very useful in setting up new models that involve both MB and the aromatic cofactor.

4. Towards physiological conditions

4.1. In vitro experiments

Since some of the aromatic compounds which

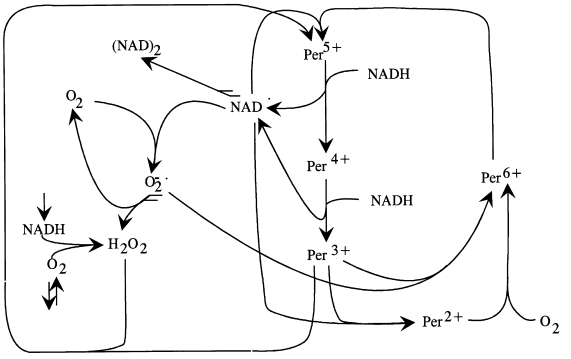

Fig. 3. Model of the PO reaction [37]. The following enzyme intermediates can be identified: Per^{3+}, ferric peroxidase; Per^{2+}, ferrous peroxidase; Per^{5+}, compound I; Per^{4+}, compound II; Per^{6+}, compound III (oxyferrous peroxidase). The reaction further involves the oxygen species superoxide ($O_2^{-\bullet}$) and hydrogen peroxide (H_2O_2) and the nicotinamide adenine dinucleotide radical ($NAD^{\bullet}$). Doubly-barbed arrows indicate that two molecules of a species are consumed. The influxes of NADH and O_2 are indicated once on the lower left by a single arrow pointing at NADH and the double arrows at O_2.

induce oscillations and complex dynamics, e.g. 4-hydroxybenzoic acid [20], are widely distributed in plants, one might wonder if such dynamics do not also occur in vivo. To investigate this possibility further we have studied the PO reaction in the presence of a number of naturally occurring phenolic compounds. Although these aromatic compounds are known to occur widely in plants [22], relatively little is known about their function. Ferulic acid is a well-known substrate for lignin synthesis [45]. 4-Hydroxybenzoic acid is known to be a cross-linking agent of the polysaccharide components of the cell wall [46] and to stimulate prostaglandin synthesis [22]. Salicylic and 4-hydroxybenzoic acids induce systemic resistance in plants [47].

Periodic oscillations of oxygen and NADH were obtained following addition of 4-hydroxybenzoic acid to a solution containing horseradish peroxidase and MB, pH 5.1. Oscillations were also obtained when repeating this experiment at pH 6.3. Table 1 summarizes the effect on the dynamics of similar additions of several phenolic compounds. Only two of the phenolic compounds studied here, vanillin and 4-hydroxybenzoic acid, showed the ability to induce sustained oscillations. According to their effect we can divide the phenols into three different categories: (1) those who induce oscillations, (2) those who do not affect the reaction and (3) those who inhibit the reaction. The latter two categories can be distinguished by adding 2,4-dichlorophenol or 4-hydroxybenzoic acid to a non-oscillating reaction mixture containing another aromatic compound. If this addition induces sustained oscillations, the phenol in question is considered to be without any effect on the dynamics. However, if the addition of 2,4-dichlorophenol or 4-hydroxybenzoic acid fails to induce oscillations the phenolic compound in question is considered to be an inhibitor of oscillations.

Table 1

Effect of various naturally occurring phenolic compounds on oscillations and complex dynamics in the PO reaction at pH 5.1 and pH 6.3

Phenol	Concentration (μM)	pH 5.1	pH 6.3
2-Methoxyphenol (guaiacol)	10–20	–	–
4-Hydroxybenzaldehyde	10–50	0	0
4-Hydroxy-3-methoxy-benzaldehyde (vanillin)	10–50	+	+
2-Hydroxybenzoic acid (salicylic acid)	100–300	0	0
4-Hydroxybenzoic acid	10–750	+	+
4-Hydroxy-3-methoxybenzoic acid (vanillic acid)	4–100	0	0
2-Hydroxycinnamic acid	5–20	0	0
4-Hydroxy-3-methoxycinnamic acid (ferulic acid)	1–10	0	–

+, Sustained oscillations; 0, no effect; –, inhibition.

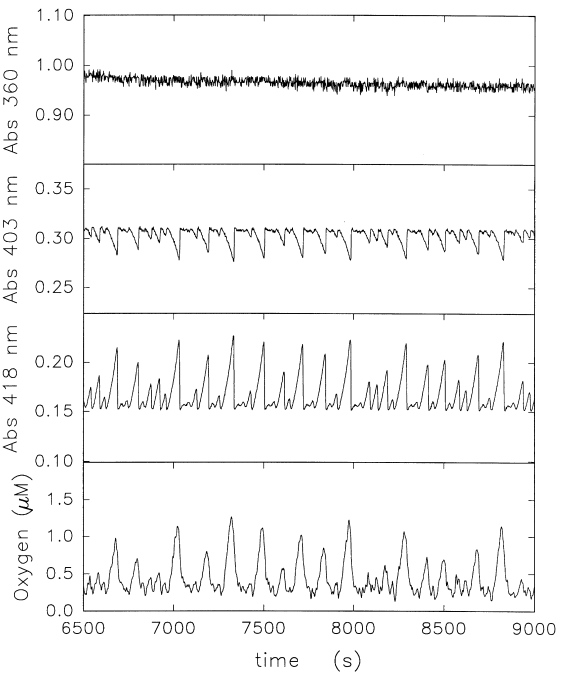

Fig. 4. Aperiodic time series of (from top to bottom) NADH, ferric peroxidase, compound III and O_2 in the presence of vanillin at pH 6.3. NADH is measured at 360 nm, ferric peroxidase is measured at 403 nm and compound III is measured at 418 nm. Experimental conditions are 1.4 μM peroxidase, 0.05 μM MB, and 30 μM vanillin. Other conditions as listed in Section 2.

As in our previous studies with DCP and other aromatic compounds [20,30] we further tested if vanillin could induce complex dynamics in addition to sustained oscillations. The results were affirmative. At pH 5.1 we observed period-doubling bifurcations, whereas at pH 6.3 we observed period-adding bifurcations and chaos as in Fig. 2. Fig. 4 shows chaotic time series of NADH, ferric peroxidase, compound III, and oxygen obtained at pH 6.3 with vanillin as a cofactor. Chaos in the time series is confirmed by an almost unimodal one-humped next-amplitude plot of the

maxima of oscillations of any of the species measured. The next-amplitude plot (not shown) of the data shown in Fig. 4 closely resembles those obtained previously with DCP [28,30] and with 4-hydroxybenzoic acid [20]. Fig. 5a shows the chaotic time series of compound III from Fig. 4 whereas Fig. 5b shows a reconstruction of the chaotic attractor made using Takens' embedding method [48] and a time delay of 6 s. We note that the attractor in Fig. 5b consists of a seemingly random mixture of large and small loops

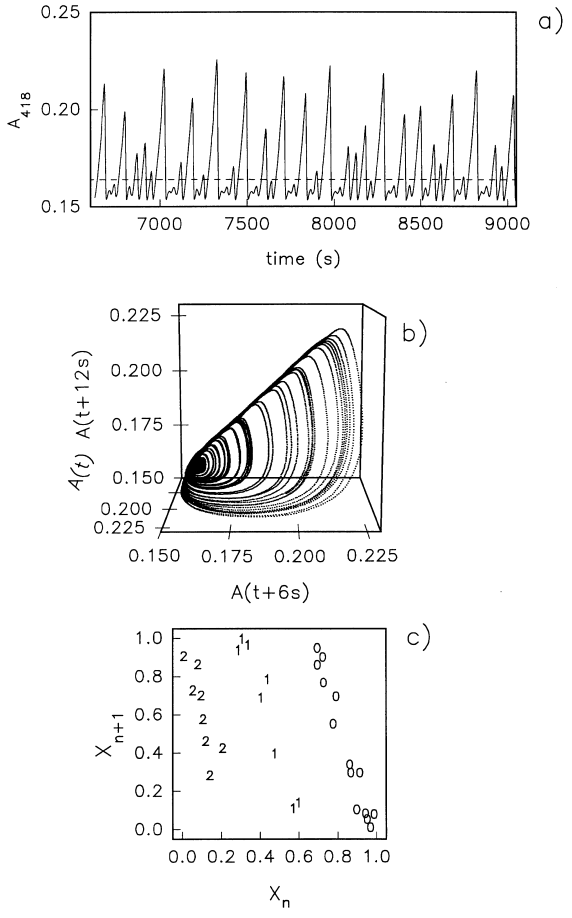

Fig. 5. Test of homoclinic chaos in the PO reaction in the presence of vanillin. (a) Time series of compound III from Fig. 4. (b) Reconstructed attractor using Takens' time delay method; (c) first return map constructed from a Poincaré section of the attractor in (b). The Poincaré section is made perpendicular to the direction of the trajectories and such that only loops of a given amplitude, as indicated by the dashed line in (a), are on the section. The numbers represent the number of small-amplitude oscillations a trajectory completes between two crossings of the Poincaré section.

around a fixed point located on the lower left of the coordinate system. The trajectory slowly winds around the fixed point in an outward spiralling mode. After a few revolutions it suddenly leaves the vicinity of the fixed point, makes one large excursion and is rapidly reinjected into the neighbourhood of the fixed point. Then the process repeats itself. Hence, the fixed point is a saddle-focus, and this opens up the possibility of a neighbouring homoclinic orbit and Shil'nikov chaos [49,50]. Such behaviour was first described theoretically for chemical reaction systems by Rössler [51]. A rigorous test for homoclinicity and Shil'nikov chaos is very difficult to perform for experimental data [52]. However, an indication for such behaviour can be obtained by constructing a Poincaré section transversal to the trajectory as it is reinjected into the neighbourhood of the fixed point [30,50]. The ensuing Poincaré map obtained from such a Poincaré section should be branched. Each of the branches is associated with the particular number of small amplitude revolutions that the trajectory has made around the saddle-focus since it last crossed the Poincaré plane. Such a plot is shown in Fig. 5c using a Poincaré section transversal to the direction of the flow, but selected such that it is intersected only by oscillations of a certain magnitude as indicated by the dashed line in Fig. 5a. The branching of this return map supports the existence of a neighbouring homoclinic orbit. A similar plot has been obtained previously with DCP as the aromatic cofactor [30].

4.2. Experiments using horseradish extracts

We investigated the dynamics of the PO reaction in cell-free extracts from horseradish roots. In such extracts several different oxidase reactions, which compete for NADH as well as for various oxygen-containing intermediates such as hydrogen peroxide and superoxide, may take place simultaneously. Thus, it is not self-evident that oscillatory dynamics should be observed in such extracts. Fig. 6a shows the dynamics resulting from starting at almost zero oxygen concentration in the liquid and quickly changing the oxygen content of the gas phase from zero to 1.05%. In this case no cofactor is present. Fig. 6b shows the same experiment, but this time MB and DCP are added as cofactors. We note that in the absence of MB and DCP the oxygen concentration

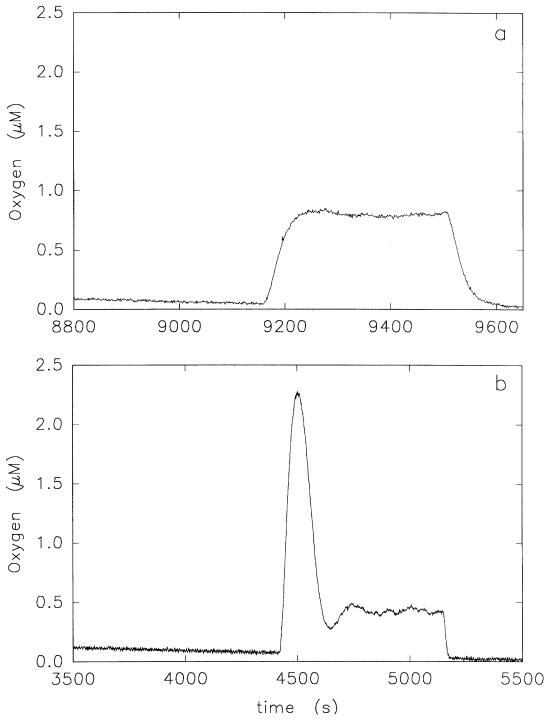

Fig. 6. Steady-state behavior (a) and damped oscillations (b) in extracts of horseradish roots. In (a) there is no phenolic compound present in the solution; in (b) 25 μM DCP is added. Experimental conditions: (a) Initially the gas phase above the extract is pure N_2 and 0.1 M NADH is supplied at a rate of 21 μl/h. At $t \approx 9150$ s the oxygen content in the gas phase is changed from 0 to 1.05% (v/v). After a further 300 s the oxygen in the gas phase is switched back to 0%. (b) Initial conditions as in (a). At $t \approx 4450$ s the oxygen content of the gas phase is changed from 0 to 1.68% (v/v). After a further 750 s the oxygen in the gas phase is again set to 0%.

approaches a steady state monotonically, while in the presence of the cofactors the steady state is approached through a damped oscillation. These experiments clearly demonstrate that extracts are capable of exhibiting oscillatory dynamics. No attempt was made to optimize the experimental conditions in order to obtain oscillatory dynamics. However, the fact that damped oscillations were observed already in our preliminary experiments suggests that a set of experimental conditions exists where sustained oscillations may be obtained. Several experiments like those shown in Fig. 6 were made in the period from November to February. The experimental results indicated that oscillatory dynamics, in the presence of MB and DCP, is obtained in horseradish root extracts prepared in late autumn, but not in extracts prepared in winter. Although we have yet to determine dynamics in extracts prepared at other seasons, these introductory experiments suggest that oscillatory dynamics are a seasonal event.

5. Discussion

The PO-reaction is known to take place in plants in vivo [23,53,54], where it uses NADH derived from the oxidation of malate [55,56]. It is generally believed that the function of the PO reaction is to produce hydrogen peroxide used in the synthesis of lignin [56,57]. However, the present experiments suggest that the reaction may have additional functions which are yet to be disclosed. We have demonstrated that phenolic compounds, which are known to be abundant in plant tissue, induce oscillatory behaviour in vitro, and that plant cell-wall extracts can show oscillatory dynamics. As for the latter our preliminary experiments only show damped oscillations, but we strongly believe that conditions can be found where sustained oscillations and complex dynamics are also observed in such extracts. It is quite likely though that oscillatory dynamics may be limited to certain times of year.

On the basis of the results presented in Section 4 and those obtained previously [20] we conjecture that oscillations and complex dynamics in the PO reaction do take place in the intact plant. At present we can only speculate about the function of such dynamics. However, based on a few examples of oscillating biochemical or biophysical processes known from mammalian physiology, some potential functions are as follows. (1) Oscillations in the PO reaction may be responsible for oscillations in the secretion of certain hormones. We base this proposal on the observation that oscillations in glycolysis in pancreatic β-cells seem to be involved in the oscillatory secretion of insulin from pancreatic β-cells [58,59]. (2) It seems to be the rule that biochemical signal-transduction systems, such as the adenylate cyclase/cyclic AMP system or the inositol trisphosphate/Ca^{2+} system, show oscillatory dynamics [1]. Some of these also show complex oscillations [60,61]. Recently, H_2O_2 and superoxide have been suggested as signal-transducing molecules [62,63]. If this is the case then peroxidase and catalase are expected to either interfere

with the signal-transduction or to be directly involved in it. Assuming that the latter is the case we have good reasons to expect simple and complex oscillations of H_2O_2 and superoxide in vivo.

In both examples mentioned above the significance of oscillatory dynamics is not yet clear. Nevertheless, the fact that oscillations are often encountered in biological processes where information is transmitted and processed could suggest that the information is contained in the oscillatory pattern itself, i.e. in its frequency and amplitude. Such frequency- and amplitude-encoded information transfer would allow a number of different information transfer systems to share a common path [64]. For example, different hormone-receptor complexes could transmit their message through a common second messenger such as cyclic AMP or calcium ion. In fact, it has been demonstrated that frequency-encoded biochemical regulation is expected to be more accurate than amplitude-encoded regulation [65].

It has been argued [1] that simple periodic oscillations are more common in nature and more advantageous to cellular processes compared to complex periodic oscillations and chaotic dynamics. These arguments were based on observations that in models of cellular processes complex oscillations and chaos are limited to a very small fraction of parameter space. However, most of these models have a small number of variables (four or less). In realistic models of enzyme reactions such as the PO reaction, many more variables are needed. In such models one finds complex dynamics and chaos in a much larger fraction of parameter space [29,37,43]. Also in our experiments with purified peroxidase we observe complex periodic oscillations and chaotic dynamics over a range of experimental conditions. For example, at pH 6.3 the experimental range in which we observe complex periodic oscillations and chaos comprises about 30% of the total range over which we observe oscillations. At lower pH this region may shrink to about 10%. Similar relations between the size of the parameter region yielding complex dynamics and the size of the region yielding oscillatory behaviour are found for the BFSO model. As for the benefit of periodic oscillations compared to chaotic dynamics it could be argued that the latter provides the organism with flexible responses to changes in the environment. This is because chaotic attractors coexist with an infinite number of unstable periodic orbits. When a system in a chaotic state is repeatedly perturbed, it can respond either by staying chaotic or by stabilizing its dynamics on one of the unstable periodic orbits in the vicinity of the chaotic attractor. Such stabilization is usually referred to as chaos control [66]. In the PO system a variety of periodic orbits have been stabilized experimentally by adequately perturbing different parameters of the reaction [32,67]. It is also worth mentioning that in one study a physiologically relevant parameter, namely the electric current, was used to control chaos [32]. Thus chaotic dynamics can be considered as a pool for an infinity of periodic motions, each of which can be stabilized when necessary.

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