

# Dynamical Instabilities in a Diffusion Layer

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We investigate the dynamics of a reaction-diffusion-convection enzyme system as a function of relevant parameters and observe reproducible types of periodic and aperiodic oscillations. These oscillations arise within a narrow diffusion layer only. Some implications for more complex reaction networks are considered.

*Key words:* Enzyme kinetics, Reaction-diffusion-convection system, Oscillation, Chaos, Diffusion layer.

## 1. Introduction

Reacting chemical and biochemical systems are one class of synergetic systems [1] where out of the action of a large number of units (i.e. molecules) macroscopic behavior arises as a new quality. For certain islands in parameter space the most simple type of attractor – a stable steady state – will lose its stability and other more complex types of attractors will appear, which lead to non-stationary behavior of the system. Interesting oscillatory results have been reported in systems where reaction kinetics and diffusion were coupled by immobilization of fructose-6-phosphate kinase, the central control enzyme of glycolysis [2]. The only experimental one-enzyme system for which temporal oscillations and chaos have been reported so far is the peroxidase-oxidase reaction [3]. Since that paper appeared in 1977 not a single artificial enzyme system has been shown to exhibit more complex behavior than simple oscillations. It has long been conjectured that spatial and temporal dissipative structures resulting from dynamical instabilities play an important role in the organization of metabolism of living systems, as well as morphogenesis and chemical evolution [4]. Several theoretical investigations have stressed the importance of feedback control for instabilities [5] and for chaos [6]. Recently the role of hydrodynamic flows as an integral part in structure formation of reaction-diffusion systems has been stressed [7]. We now designed a new model system based on a one-enzyme reaction with feedback control of one of

the products which couples reaction-diffusion processes to well-controlled laminar convection. We present a parameter study of this system showing a far-from-equilibrium steady state as well as sustained periodic and chaotic oscillations. Due to the experimental set-up it can be shown that a dissipative structure arises in an extremely narrow diffusion layer supporting the view that such structures might be generated within a single living cell. We discuss the value of this artificial system as an experimental model for the dynamical organisation of biochemical reactions.

## 2. Experimental

The set-up used was as described previously [8]. A temperature difference at the surface of the solution was kept constant at  $\Delta T = 1.2^\circ\text{C}$  for all experiments to add a laminar convective flow in front of the electrode. The working electrode was a glassy carbon electrode with a diameter of 2 mm; all potentials given are vs. SCE. The solution contains hydrogen peroxide, benzoquinone and horseradish peroxidase in an acetate buffer of pH = 5.0 (cf. Fig. 1). There is no hydroquinone present in the beginning, it is synthesized at the electrode only after application of a negative potential. It then starts to spread due to diffusion and is consumed by reaction with the enzyme to yield benzoquinone, which can be reduced at the electrode again, and so forth. The enzyme reaction is fast and irreversible; so at some short distance from the electrode there will effectively be no hydroquinone at all.

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### 3. Results

To study the system we varied parameters and recorded the system's dynamical response as a function of time. Figures 2 a–c show a sequence of chronoamperometric experiments at a constant potential with increasing initial concentration of hydrogen peroxide  $[\text{H}_2\text{O}_2]_0$ . These three parameter values have been selected to demonstrate three distinct types

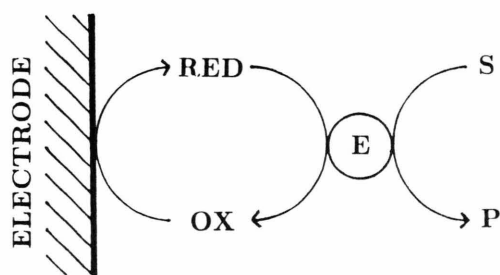


Fig. 1. Principle of reaction of a substrate S and a second substrate RED (cosubstrate) catalyzed by an enzyme E (yielding products P and OX) with an electrochemical feedback loop to recover the reduced form of cosubstrate by reduction of OX at the surface of an electrode. In our case:  $\text{S} = \text{H}_2\text{O}_2$ ,  $\text{RED} = \text{Hydroquinone}$ ,  $\text{E} = \text{horseradish peroxidase}$ ,  $\text{P} = \text{H}_2\text{O}$ ,  $\text{OX} = 1,4\text{-benzoquinone}$ .

of dynamical response to the applied potential, which is in the range where the uncatalyzed electrode reaction would yield a limit current. Figure 2a is a stable steady state; the current is catalytically enhanced compared to the noncatalyzed system. The electrode and the enzyme have cooperatively balanced their turnover and the whole arrangement is stable with respect to small external disturbances. This is a typical steady state far from equilibrium. The steady state current is approximately  $30 \mu\text{A}$  compared to  $2\text{--}3 \mu\text{A}$  in the absence of enzyme. Looking at Fig. 2c now, we indeed see sustained oscillations of the current with only weak damping. After about three hours the oscillation suddenly becomes strongly damped and the system soon ends up in a steady state. This suggests that the weak damping is the result of either accumulation of an interfering product or is due to slow consumption of hydrogen peroxide. The latter possibility can be excluded as being the only reason, because simply decreasing the initial concentration for the experiment does not suffice to observe the previous sharp transition into a steady state. As Fig. 2b shows for an intermediate value of  $[\text{H}_2\text{O}_2]_0$  we find a new dynamical regime of lower average frequency and smaller average amplitude, with the dynamics being aperiodic in all experiments performed. The third

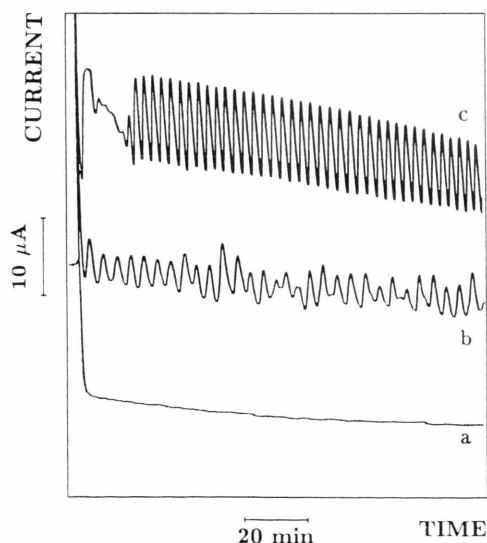


Fig. 2. Time series of current response at a constant potential of  $-300 \text{ mV}$  and a constant temperature gradient of  $1.2^\circ\text{C}$ . Initial concentrations are:  $[\text{benzoquinone}]_0 = 4 \text{ mM}$ ,  $[\text{peroxidase}]_0 = 0.21 \mu\text{M}$ . a)  $[\text{H}_2\text{O}_2]_0 = 2 \text{ mM}$ , b)  $[\text{H}_2\text{O}_2]_0 = 2.5 \text{ mM}$ , c)  $[\text{H}_2\text{O}_2]_0 = 3.0 \text{ mM}$ . See [8] for details.

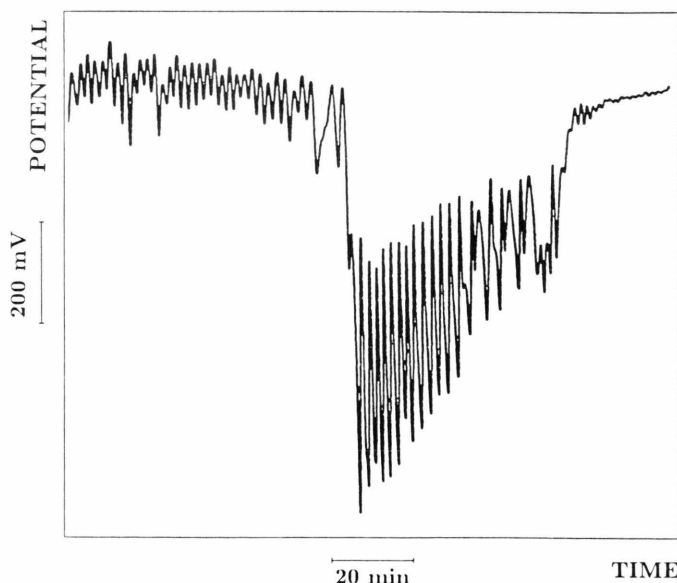


Fig. 3. Time series of potential response at a constant current of  $60.1 \mu\text{A}$ . Other parameters as in Figure 2c.

regime appears stable and reproducible at the given conditions. We thus can assume that in the case of an ideal system with no irreversible side reactions and with a constant concentration of  $\text{H}_2\text{O}_2$  inside the “stock” solution there exists a chaotic attractor for the system. Interestingly the latter regimes is not stable for arbitrarily long times. For example, a system started out at 3.0 mM  $\text{H}_2\text{O}_2$  and  $\Delta T = 1.6^\circ\text{C}$  begins with chaotic behavior, but after approximately one hour switches to large amplitude single-peak oscillations similar to Figure 2c. The chaotic result of Fig. 2b, too, does not simply decay into a steady state, but also eventually switches to a short-lived periodic behavior followed by a stable steady state. The amplitude of the latter oscillation is small compared to that in Figure 2c. Nevertheless there is no doubt that both oscillations are related because experiments performed at intermediary parameter values show that the transition occurs continuously. Furthermore, extensive search in parameter space suggests that the low frequency oscillations of the induction period in Fig. 2b may belong to yet another regime (probably a small amplitude limit cycle) which then bifurcates into chaos. A further chaotic result is found if under the same set of initial conditions a constant current is allowed to pass through the reaction cell instead of a constant potential being applied. In this case the potential is recorded as a system variable. Figure 3 is an example at  $i = -60.1 \mu\text{A}$ . It apparently demonstrates the phenomenon described above, namely, the switching from one dynamical state to another. Our system obviously possesses several subsets, namely, an aperiodic regime of small amplitude, an oscillation with large amplitude and a mixed type of behavior, composed of two-peak oscillations. It cannot yet be derived from these first results whether the corresponding attractors are successively being created through bifurcations around an unstable steady state, or whether changes in parameters nudge the system from one basin of attraction into another in a state space with several coexisting attractors. None of several perturbation experiments tried (brief variations of applied potential and rotation of electrode) succeeded in changing the dynamics of the system. Also we found no hysteresis.

#### 4. Discussion

Our investigation revealed that the catalytic steady state of a specific reaction-diffusion-convection system

can lose its stability. We found two types of almost regular oscillatory behavior and an intermediary regime with chaotic behavior. The set-up did not allow to study attractors for arbitrarily long times; rather we observed sequences of dynamical regimes with all external parameters kept constant. In biochemistry a sequence of qualitatively different regimes might well constitute an important feature in the control of cellular metabolism. Slow changes of parameters will always be present in real systems, in the form of side reactions, a changing substrate supply, or variations of the surrounding medium. For example, cell parameters in living systems are frequently subject to circadian rhythms (a uniform slow change on the time scale of chemical oscillations). In that case the response of a system like the one introduced would be bound to switch between qualitatively different states. It is conceivable that some day different types of dynamical response may be assigned specific role in the molecular processing of the information flowing into a cellular system from the outside. Our experiments have shown that a dissipative structure can arise within a narrow diffusion layer. Using the approximation of a linear Nernst layer we calculated a thickness of  $20 \mu$  for a steady state current of  $60 \mu\text{A}$  and an estimated diffusion coefficient of  $1 \cdot 10^{-5} \text{ cm}^2 \cdot \text{s}^{-1}$ . Thus our results suggest that dissipative structures might arise as structured units on the level of a single living cell. Intracellular or intercellular coupling of such units would then result in structure formation on a higher level. Keeping in mind that already a single four variable well-stirred system is capable of generating hyperchaos [9], unforeseen complexity in space and time might arise from spontaneous selforganization in single cells as well as in tissues. As far as potential applications are concerned, our system also represents a whole new class of dynamically interesting systems. The principle of an electrochemical feedback loop employed in Fig. 1 can also be applied to the regeneration of NADH [10]. In consequence, a large number of reactions may be investigated concerning the stability of their catalytic steady state.

#### 5. Summary

Our intention has been to present a system with a highly complex dynamics not only for its intrinsic dynamical interest, but also to thereby stimulate further abstract experimental modelling of the coupling features relevant in enzyme-catalyzed reactions com-

binning nonlinear kinetic regulation, feedback loops of metabolic intermediates, the interplay of localized and homogeneous reactions and the additional feature of a convectional flow. Any attempt to understand the organization of cellular metabolism should start with an insight into what types of dynamical instabilities are possible, at least in principle, in such systems. So far there is no explicit system of coupled differential equations to explain the results. Based on the experiments one might pose the question of whether the concept of a stable attractor is an appropriate tool for the description and characterization of such a highly

complicated (in terms of differential equations) system. It will be interesting to see whether a classification of a system with more than a limit cycle attractor could be achieved in terms of sequences of attractors in the ODE limit. Such an investigation might be of advantage to consult when trying to observe living systems, where we might indeed never be able to experimentally fully separate all attractors for individual investigation without losing the object's main properties.

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