Instabilities in a Simple Enzyme Reaction Caused by pH-Dependence*

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We investigate the model of an enzymatic reaction with Michaelis-Menten kinetics and bell-shaped pH-dependence of the reaction rate. In the case of proton consumption the reaction can generate oscillations in a homogeneous reactor and turbulent spatio-temporal patterns in a reaction-diffusion environment.

1. Introduction

The idea that "many metabolic reactions work beyond the transition point corresponding to the appearance of a dissipative structure" [1] sounds appealing. One should therefore expect that it should be possible to observe dynamic instabilities of enzyme reactions *in vitro*. Indeed, many models of enzymatic reactions have been shown to oscillate. Experimentally, however, so far only one homogeneous single-enzyme reaction, the peroxidase-oxidase reaction, was found to exhibit sustained oscillations in an open reactor [2]. We investigate the question whether commonly occurring features of enzyme reactions can lead to dynamic instabilities under realistic assumptions and develop a prototypic model for temporal and spatio-temporal instabilities.

2. The model

The reaction of a substrate S producing P catalyzed by enzyme E

$$\mathrm{S} \xrightarrow{\mathrm{E}} \mathrm{P}$$

can be described by saturation kinetics of the reaction rate

$$v = k' \epsilon_0 S/(k_M + S), \tag{1}$$

where S is the concentration of S, k' is the rate constant, ϵ_0 denotes the enzyme concentration, and k_M is the Michaelis constant. This kind of reaction is unable to undergo a dynamic instability when run in a continuous flow experiment. There are two simple extensions of this kinetic unit which do allow the possibility of an instability:

$$\mathrm{I} + \mathrm{S} \xrightarrow{\mathrm{E}} \mathrm{P}$$

where I is an inhibitor of the reaction, i.e. an increasing concentration of I decreases the reaction rate; and

$$\mathrm{S} \xrightarrow{\mathrm{E}} \mathrm{P} + \mathrm{A}$$

where A is an activator of the reaction, i.e. an increasing concentration of A increases the reaction rate.

Such situations can be realized if an enzyme with a bell-shaped dependence of the reaction rate on the pH either consumes or produces protons during the reaction. As bell-shaped pH characteristics are widely found in enzyme kinetics, a large number of reactions are possible candidates for the search for instabilities in single-enzyme systems. Here, we consider the case of a proton consuming reaction

$$\mathrm{H^+} + \mathrm{S} \xrightarrow{\mathrm{E}} \mathrm{P}.$$

The kinetics of such a reaction can be described in a first approximation for the bell-shaped pH-dependence as follows:

$$\begin{aligned} \dot{S} &= a_1 - k' \epsilon_0 S H/(k_M + S + d H^2) - b_1 S, \\ \dot{H} &= a_2 - k' \epsilon_0 S H/(k_M + S + d H^2) - b_2 H, \end{aligned} \tag{2}$$

where H denotes proton concentration, d is a parameter, a_1 and a_2 are constant inputs, and b_1 and b_2 are

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constants for first-order reactions or first-order outflows from the reactor. Note that due to the combination of the shape of the pH-curve and the consumption of protons, the proton concentration can have two opposite effects. As long as the reaction rate

$$v = k' \epsilon_0 S H/(k_M + S + d H^2) \quad (3)$$

increases with H (the "alkaline flank" of the function), protons act as activator with respect to the reaction rate. However, the assumed consumption of protons leads to a negative effect on $\dot{S}$ and $\dot{H}$. Thus, in this case protons are inhibitor with respect to the differential equation (2). As long as v decreases with H (the "acidic flank" of the function), protons act as inhibitor with respect to the reaction rate, Here, the assumed consumption of protons leads to a positive effect on $\dot{S}$ and $\dot{H}$. Thus, in this case protons are activator with respect to the differential equation (2).

The reaction is assumed to take place in a continuous flow stirred tank reactor (CSTR). Substrate, enzyme, and proton-containing buffer are pumped continuously into the well-stirred reactor. There is an overflow mechanism and a temperature bath to keep volume and temperature of the reactor constant. For constant concentration of protons (well-buffered solution), (2) reduces to (1).

Models of this type were investigated with minor modifications by Seelig [3] and Thomas [4]. However, we would like to point out that such a model can be obtained quite generally in proton-consuming reactions with bell-shaped dependence of reaction rate on pH. We take realistic values for the parameters $k = k' \cdot \epsilon_0$, k_M and d, and study the occurence of instability as a function of experimentally accessible parameters a_1 and a_2. For simplicity we take $b_1 = 0$ and $b_2 = 1$. This leads to the following system of coupled differential equations for a proton-consuming single-enzyme reaction:

$$\begin{aligned} \dot{S} &= a_1 - k S H/(k_M + S + d H^2), \\ \dot{H} &= a_2 - k S H/(k_M + S + d H^2) - H. \end{aligned} \quad (4)$$

3. Results

Limit cycle oscillations

Figure 1 shows the overall reaction rate v according to (3) for a chosen set of parameters. The shape

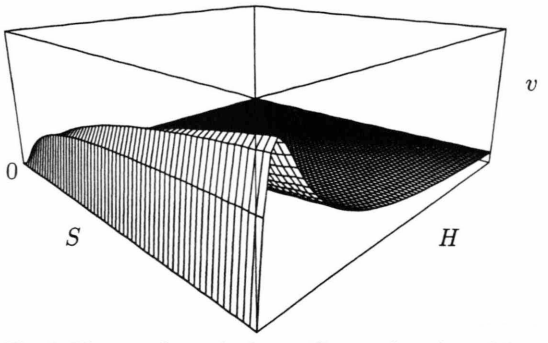

Fig. 1. The reaction velocity v, (3), as a function of the concentration of substrate S and the concentration of protons H. Parameter values: $k = 1000$, $k_M = 10^{-5}$, $d = 1.3 \cdot 10^6$. The origin is the lower left corner of the box marked 0. Axes: $0 < v < 0.015$, $0 < H < 0.0005$, $0 < S < 0.015$.

of the function combines the optimum function for the dependence on proton concentration and the saturation function for the dependence on substrate concentration. Either diagonal element of the Jacobian matrix of (4) evaluated around the steady state, i.e. $(\partial \dot{H}/\partial H)_{ss}$ and $(\partial \dot{S}/\partial S)_{ss}$, has a negative sign on the alkaline flank of v. Thus no instability is possible. On the acidic flank of v the diagonal element of the Jacobian corresponding to protons can be positive, whereas the diagonal element corresponding to substrate remains negative. It is this latter case which can lead to an instability of the steady state of the reaction. The trace of the Jacobian can change signs and thus with complex eigenvalues the system can undergo a Hopf bifurcation.

The fixed point of the system (4) is given by

$$\begin{aligned} S_{ss} &= [a_1 (k_M + d (a_2 - a_1))]/[k (a_2 - a_1) - a_1] \\ H_{ss} &= a_2 - a_1 \end{aligned} \quad (5)$$

We keep the fixed point H_{ss} constant, $(a_2 - a_1) = 0.00002$, and take a_1 as bifurcation parameter.

The fixed point is stable for small values of a_1 (e.g. $a_1 \leq 0.0008$, with all other parameters as in Figure 1). As a_1 is increased the fixed point undergoes a Hopf bifurcation ($a_1 \approx 0.00085$). The fixed point is unstable for parameter values beyond this point. We find oscillations around the unstable steady state. The oscillations are harmonic with small amplitude near the Hopf bifurcation and become relaxational with increasing amplitude as the parameter a_1 is increased. A relaxation oscillation consists of a fast spike in both H and S, and a phase with slowly decreasing substrate concentration at (almost) constant minimum pH.

Diffusion-induced chaos

Now we consider two diffusively coupled oscillators (4):

$$\begin{aligned}
\dot{S}_1 &= a_1 - \frac{k\,S_1\,H_1}{k_M + S_1 + d\,H_1^2} + D_S\,(S_2 - S_1),\\
\dot{H}_1 &= a_2 - \frac{k\,S_1\,H_1}{k_M + S_1 + d\,H_1^2} - H_1 + D_H\,(H_2 - H_1),\\
\dot{S}_2 &= a_1 - \frac{k\,S_2\,H_2}{k_M + S_2 + d\,H_2^2} + D_S\,(S_1 - S_2),\\
\dot{H}_2 &= a_2 - \frac{k\,S_2\,H_2}{k_M + S_2 + d\,H_1^2} - H_2 + D_H\,(H_1 - H_2).
\end{aligned} \tag{6}$$

Compared to (4) there are two new parameters, namely, the diffusion coefficient of protons, D_H, and the diffusion coefficient of substrate, D_S.

For a given value of a_1 and a_2 in the oscillatory region it is possible to find a coupling strength where the two oscillators show phase instability and the resulting dynamics takes place on a two-torus or a chaotic attractor. The fact that chaos can arise (e.g. for $a_1 = 0.001$, $a_2 = 0.00102$, $D_S = 0.06$, $D_H = 0.6$, other parameters as in Fig. 1) is of importance because this is a first hint that a turbulent regime may be possible in the partial differential equation of a continuous reaction-diffusion system (see [5, 6]).

Spatiotemporal instabilities

We consider a continuous reaction-diffusion system in one spatial dimension introducing diffusion terms to the oscillator equation (4). This system is implemented for integration by the method of finite differences using N coupled two-variable reaction cells given in (4). For large N we assume the solution of the coupled system to converge towards the solution of the continuous reaction-diffusion system. As in the preceding section, D_H will be realistically assumed to be ten times larger than D_S.

With zero-flux boundary conditions and the choice $D_S = 1.11$, $D_H = 11.1$, $a_1 = 0.001$, $a_2 = 0.00102$ (other parameters as in Fig. 1) and random initial conditions we found a stable weakly turbulent regime for $N = 100$. The behavior is irregular in time and space, and there are randomly moving phase dislocations (phase defects) along the system. Figure 2 shows a space-time plot of a simulation after transients have died out. The turbulent regime does not vanish if N is increased further. This behavior is stable with respect to perturbations and related regimes can be found for other sets of parameters as well. If we assume the real diffusion coefficient of protons to be 10^{-5} cm^2 s^{-1} then the spatial extension of Fig. 2 corresponds to a length of 1 mm.

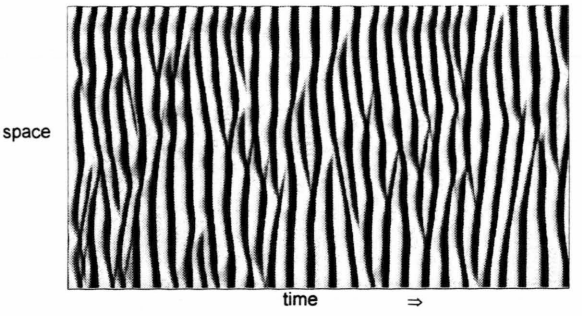

Fig. 2. Turbulent spatio-temporal regime in 100 diffusively coupled cells (4). Parameter values: $D_S = 1.11$, $D_H = 11.1$, $a_1 = 0.001$, $a_2 = 0.00102$. Other parameters as in Figure 1. Δ-time = 100. Zero-flux boundaries (compare (5)).

4. Discussion

We have studied a simple two-variable model for the kinetics of a proton-consuming enzymatic reaction. The model combines a saturation function for the velocity of substrate turnover and a bell-shaped dependence of reaction rate on the pH of the solution. On the acidic flank of the rate function (see Fig. 1) the consumption of protons leads to an acceleration of the reaction rate. This effect can be exploited to generate an instability of the fixed point of the system. The instability leads to limit cycle oscillations for realistic sets of parameters. A similar oscillation can be found on the alkaline flank of the pH function if a proton-generating reaction is considered. Of course, the effectors of the enzymatic reaction rate need not be protons. However, a bell-shaped dependence of reaction rate on pH is so common a phenomenon, e.g. with proton-consuming oxidoreductases, that it offers the possibility for a general mechanism to create an instability in metabolic reactions. With these results in mind, kinetic data from the literature may now be used to predict oscillations for specific reactions in a CSTR.

As is known from previous studies [5] diffusive coupling of two oscillating reactors may lead to toroidal oscillations and chaos. We easily found these quasiperiodic and aperiodic solutions in our model for medium coupling strength between the reactors. Very

weak and very strong coupling both result in synchronized behavior of the two-cell system. If the oscillator equation (4) is viewed as an activator-inhibitor system in the sense of Turing [7] then the protons act as activator and the substrate acts as inhibitor. Thus the requirement for time-independent Turing structures ($D_I \gg D_A$) cannot be fulfilled for a substrate with a lower diffusion coefficient than that of protons.

Nevertheless, the difference in diffusion coefficients between substrate and protons causes complex spatio-temporal symmetry breaking and self-organization of the reaction-diffusion system. Our model calculations suggest a scale of one millimeter for which these structures may occur but a turbulent regime could be detected with different parameters on smaller scales as well. Again, introduction of specific kinetic parameters will allow prediction of turbulent patterns for *in vitro* experiments.

Despite of many speculations there seems to be no clear evidence so far which function a given dissipative structure may serve in metabolism. It is improbable that a turbulent regime as shown in Fig. 2 can be controlled sufficiently to stay on its attractor for a long time *in vivo*. Rather, one would expect that it will appear as a transient between two more coherent states if an external parameter varies continuously or periodically. A far-reaching consequence of the spatio-temporal instabilities in our model is the fact that the system describes the pH of the solution. The oscillations can change the pH significantly. This, of course, will effect all other enzymatic reactions in the surroundings, particularly those with a sharp pH optimum and those which work on a flank of their pH curve. Local pH fluctuations of one reaction would thereby strongly effect the whole metabolism within an area. Sustained pH oscillations were predicted for a coupled system involving two michaelian enzymes, and damped oscillations were observed experimentally in a system with proton-producing alcohol dehydrogenase and proton-consuming alanine dehydrogenase [8]. In this case, however, it was crucial for the oscillations to occur that some species (e.g. NADH) was cyclically generated and consumed by the two enzymes.

Finally, as a consequence of our numerical results we suggest to experimental investigators of self-organized enzymatic reactions *in vitro*:

- focus on the flank rather than the peak of the pH curve;
- do buffer insufficiently rather than work at constant pH during the reaction; and
- allow diffusive coupling in addition to well-stirred solutions and measure a reaction variable continuously as a function of space and time.

The observation of coherent and turbulent spatio-temporal instabilities during an *in vitro* enzyme reaction should be within reach of the experimentalist.

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