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Threshold Excitations, Relaxation Oscillations, and Effect of Noise in an Enzyme Reaction

(multiple stationary states/quasi-periodic behavior)

H.-S. HAHN, A. NITZAN, P. ORTOLEVA, AND J. ROSS

Department of Chemistry, Massachusetts Institute of Technology, Cambridge Mass. 02139

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ABSTRACT **We study a deprotonation reaction by an enzyme with activity dependent on pH. The rate and transport equations are simplified with a number of assumptions, are analyzed according to the presence of different time scales, and are solved numerically to show relaxation oscillation and threshold excitation, for different choices of parameters. The imposition of fluctuations (noise) on the deterministic equations for threshold excitation conditions leads to random occurrence of an excitation and return to steady state at low noise level and to large, random variations in concentrations at high noise level. At intermediate noise levels (of the order of the threshold excitation), however, we find quasi-periodic concentration oscillations. Thus, critical values of external constraints necessary for oscillations are altered by the presence of noise.**

I. Introduction

Certain chemical reaction mechanisms (possibly coupled with transport) in systems maintained far from equilibrium are known to have nonlinear properties such as multiple steady-states, limit cycles, relaxation oscillations, and steady-states with threshold excitations (1–3). In this article we study one such mechanism, chosen and simplified with some arbitrariness. The mechanism has multiple time scales and we take advantage of that fact. We obtain numerical solution of the rate and transport equations and find the cited nonlinear properties for suitable choices of the parameters. We use the mechanism then, under conditions for threshold excitations, to investigate numerically the effect of the imposition of noise, that is fluctuations, on the deterministic equations. We find the interesting possibility of quasi-periodic motion for a system in a steady state subject to threshold excitation. This occurs at noise levels comparable to the threshold. For lower noise levels the threshold excitation occurs randomly, and for higher noise levels concentration variations become again more random.

We consider the following system: A substrate S is deprotonated by an enzyme EH_2 (products P^-, H^+) within an enclosure. The enzyme may dissociate to the inactive forms EH^-, $E^=$, and hence the catalytic strength of the enzyme is pH dependent; enzyme in any form is confined to the enclosure which is surrounded by fixed concentrations of reactants and products.

In a similar system (hydrolysis of benzoyl-L-arginine ethyl ester catalyzed by the enzyme papain which is localized in a membrane), Naparstek, Thomas and Caplan (4) observed periodic variations of pH and stressed the importance of diffusion (or permeation) in enzyme reactions. This result was reproduced qualitatively with a computer simulation by Caplan, Naparstek and Zabusky (5). Homogeneous enzyme catalysis with inhibition and reactivation were studied by Karfunkel and Seelig (6) for the purpose of showing the possibility of relaxation oscillations and threshold excitation for such reactions. The complexity of the reaction mechanism necessary for given types of nonlinear behavior is decreased if the catalyst is localized, in a membrane for instance, and permeation into and out of the membrane plays a role [see Hahn, Ortoleva, and Ross (7)]. Bunow (8) investigated the occurrence of multiple steady-states in model enzyme reactions confined to an enclosure, and compared such systems to "continuous flow stirred tank reactors" (9). In addition to the nonlinear phenomena already mentioned, he discusses homeostatic regulation, amplification, and irreversible differentiation. For a discussion of a similar model system in relation to irreversible transitions, see (7) and for a asymmetric cell differentiation see Ortoleva and Ross (10, 11).

An analysis of excitation and threshold phenomena in the transmission of signals in nerves was made by FitzHugh (12). He used as a mathematical model a modified two-variable van der Pol equation, called BVP model (Bonhoeffer-van der Pol equation), and correlated various nerve phenomena with predictions of that model. There are close similarities in the topology of the phase plane and qualitative predictions between the BVP model and the present work.

II. System and dynamics

We consider the reactions

$$EH_2 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EH^- + H^+, \qquad [1.1]$$

$$EH^- \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} E^= + H^+, \qquad [1.2]$$

$$S + EH_2 \xrightarrow{k_3} P^- + H^+ + EH_2 \qquad [1.3]$$

$$H_2O \underset{k_{-4}}{\overset{k_4}{\rightleftarrows}} OH^- + H^+, \qquad [1.4]$$

to occur homogeneously at constant temperature and pressure, in a fixed volume V with a surface area A. The pH-dependence of the enzyme activity comes from the first two reactions (13). The deprotonation step is assumed to be irreversible.

Abbreviations: rms, root mean square; BVP model, Bonhoeffer–van der Pol model.

Since EH_2 is taken to be the active form of the enzyme, the system has a nonlinear positive feedback character; the progress of the deprotonation reaction favors the formation of EH_2 which in turn catalyzes the deprotonation reaction.

The analysis of the reactions and permeations across the boundary of the volume is facilitated by the following considerations:

(*a*) The flux of water, J_{H_2O}, although present, is neglected and the concentration of water is assumed to be constant.

(*b*) Fluxes of neutral species, e.g., S, are taken to be proportional to the concentration difference across the boundary membrane,

$$J_S = h_S (S^0 - S), \qquad [2]$$

where h_S is the permeability constant of S multiplied by the ratio of surface to volume (A/V). The superscript ^{0} denotes an external quantity.

(*c*) There exist two time scales in the system. For the shorter time scales we introduce a smallness parameter ϵ. These time scales include that of the attainment of electroneutrality and the equilibrium of the reactions (enzyme − H) and (OH − H). The slow processes then are the deprotonation of the substrate S and the transport of materials across the membrane.

(*d*) On the long-time scale we impose a condition (frequently used in experiments) of zero current clamp. This implies that the fluxes of ionic species are due to the transport of neutral pairs (H, P), (H, OH), or by exchange of OH and P across the boundary. This provision guarantees the electroneutrality of the system. Thus we have

$$J_H = J_P + J_{OH} \qquad [3]$$

Next we assume the simplest form for J_P

$$J_P = h_P (P^0 - P). \qquad [4]$$

thus neglecting cross-coupling and nonlinear effects.

These considerations yield the following equations of change:

$$\frac{d}{dt}(EH_2) = \frac{1}{\epsilon}(-W_1), \qquad [5.1]$$

$$\frac{d}{dt}(EH) = \frac{1}{\epsilon}(W_1 - W_2), \qquad [5.2]$$

$$\frac{d}{dt}(E) = \frac{1}{\epsilon}(W_2), \qquad [5.3]$$

$$\frac{d}{dt}(H) = \frac{1}{\epsilon}(W_1 + W_2 + W_4) + W_3 + J_H, \qquad [5.4]$$

$$\frac{d}{dt}(S) = -W_3 + J_S, \qquad [5.5]$$

$$\frac{d}{dt}(P) = W_3 + J_P, \qquad [5.6]$$

$$\frac{d}{dt}(OH) = \frac{1}{\epsilon}(W_4) + J_{OH}, \qquad [5.7]$$

$$\frac{d}{dt}(H_2O) = \frac{1}{\epsilon}(W_4). \qquad [5.8]$$

The symbols W_1, etc., denote the net chemical rates of reaction [1.1], etc. Processes which occur on the fast time scale are noted by the factor $1/\epsilon$ in the rate laws. The fact that enzyme species do not permeate and are conserved is seen by adding the first three equations of [5].

We want to solve the set of equations [5] to lowest order in the limit $\epsilon \to 0$, which amounts to the steady state approximation in chemical kinetics (14).

To this order we obtain

$$\frac{dS}{dt} = -k_3 \cdot EH_2 \cdot S + h_S (S^0 - S), \qquad [6.1]$$

$$\frac{dH}{dt} = [k_3 \cdot EH_2 \cdot S + h_P(P^0 - P)] \Big/ \left(\frac{dP}{dH}\right). \qquad [6.2]$$

For solutions to higher order it is necessary to use singular perturbation theory (15).

III. Steady-states and nonlinear oscillations

A. Steady-States. For a range of parameters three steady-states are possible and the system shows typical hysteresis phenomena as, for example, S^0 is varied; at an upper and a lower transition value of S^0, the system makes a discontinuous jump from one branch of steady states to another (7, 16, 17). Transitions induced by variations of external P^0 may be irreversible for certain ranges of fixed S^0 (7).

B. Relaxation Oscillations. In many observed cases of biochemical and physicochemical oscillations (18–20), it is found that during each period of an oscillation a relatively smooth phase is followed by a rapid variation of concentration occurring on a much shorter time scale. Such chemical relaxation oscillations have been studied (6, 22) for model reacting systems.

We expect that in the present system, relaxation oscillations may occur under circumstances when initially the substrate slowly permeates into the system and the internal H is sufficiently low, such that the enzyme activity is essentially negligible. When subsequently a high substrate level is attained by permeation, the H concentration rapidly increases due to the nonlinear feedback until the enzyme activity becomes appreciable. Then, the substrate is eliminated, and finally the system is flushed of H by an assumed rapid (H, P) permeation. The conditions favoring this sequence of events are large S^0 and h_P with all other rate constants of order unity. To achieve these conditions we introduce into [6] the definitions:

$$\mathcal{S} \equiv S/S^0, \quad \eta = h_P/S^0; \qquad [7]$$

on eliminating time from [6], we obtain

$$\frac{d\mathcal{S}}{dH} = \frac{1}{S^0} \frac{-k_3 \cdot EH_2 \cdot \mathcal{S} + h_S(1 - \mathcal{S})}{[k_3 \cdot EH_2 \cdot \mathcal{S} + \eta(P^0 - P)]/(dP/dH)}, \qquad [8]$$

which is to be solved in the limit of large S^0 and h_P ($= \eta S^0$).

At large S^0, the trajectories (solutions to [8]) are at almost constant $\mathcal{S}$ everywhere except on contours where the denominator of [8] vanishes (22). Contours of $d\mathcal{S}/dt = 0$ and $dH/dt = 0$ are shown in Fig. 1*a* for a given set of parameters. The stability of the steady-state is determined by the value of S^0 at fixed values of the other parameters.

Beyond a critical value of S^0, the systems develop a limit cycle which as S^0 approaches infinity, becomes a relaxation oscillation; see Fig. 1*b*. For this limiting case, during one oscillation period, $\overline{BC}$ and $\overline{DA}$ correspond to the fast processes and are traversed in a negligible time whereas $\overline{AB}$ and $\overline{CD}$ correspond to the slower processes; see Fig. *1a*.

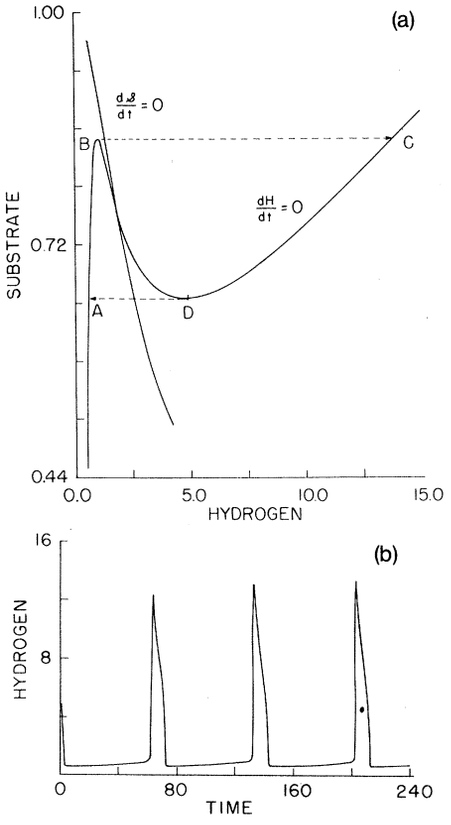

FIG. 1. (*a*) Phase plane diagram (substrate versus hydrogen ion concentration) showing a trajectory (ABCDA) of the solution to [8] in the limit $S^0 \to \infty$. Solid lines denote $dS/dt = 0$ and $dH/dt = 0$. Parameters chosen for illustration are: $K_1 = 10$, $K_2 = 3$, $k_3 = 1$, $K_W = 10^{-2}$; $h_S = 1/50$, $\eta = 1/300$; $P^0 = 0.2$, $E^0 = 0.1$. The time is in seconds and the concentration in μmol/liter. In the following figures, the parameters are the same unless specified otherwise. Fig. 1(*b*). Relaxation oscillation of H concentration versus time when $S^0 = 10^4$. The periods τ for various values of S^0 are: $\tau(S^0 = \infty) = 62.0$; $\tau(S^0 = 10^4) = 70.0$; $\tau(S^0 = 10^3) = 92.0$.

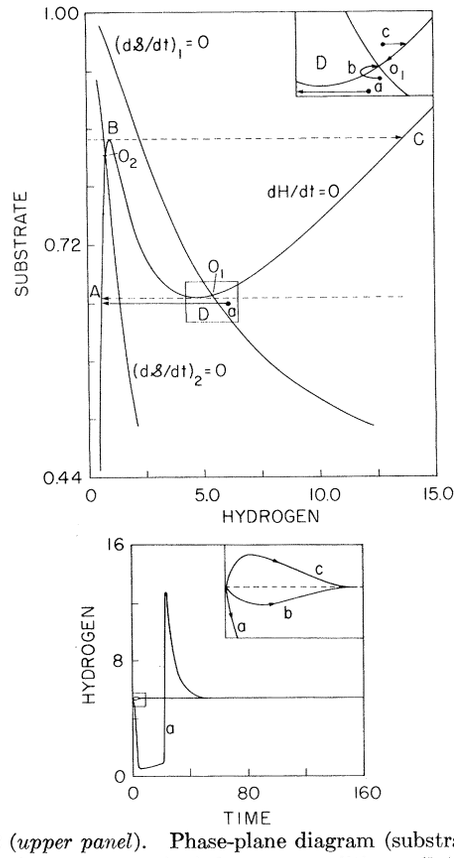

FIG. 2 (*upper panel*). Phase-plane diagram (substrate versus hydrogen ion concentration) for two conditions, $(h_S)_1 = 1/20$ and $(h_S)_2 = 1/125$. Each condition yields a stable steady state (O_1, O_2, respectively) with the possibility of threshold excitation. Perturbation of the system in O_1 to threshold excitation, point *a* on separatrix (broken line AD) or beyond, causes an excitation (trajectory $aABCO_1$) and return to O_1. Perturbations from O_1 to points *b*, *c* (*inset*) do not cause excitations. A similar situation holds if the system is in O_2, except that the threshold excitation follows an increase in substrate from O_2 past the separatrix (broken line BC).

(*lower panel*). Responses of the system (hydrogen ion concentration versus time) when subject to perturbations from stable steady state O_1 to points *a*, *b*, *c*, see Fig. 2 (upper panel). The inset gives details near zero time.

If S^0 is less than the critical value and the steady state is stable, then the trajectories show damped oscillations and eventually spiral into the steady-state. Thus, a perturbation from the steady state leads in this case to trains of consecutively damped pulses.

One also can calculate the period τ of the oscillation in the limit $S^0 \to \infty$. From [6] and [7] we see that

$$\tau = \oint dS/[-k_3 \cdot EH_2 \cdot S + h_S(1 - S)]. \qquad [9]$$

However, the portions $\overline{BC}$ and $\overline{DA}$ do not contribute to τ and on portions $\overline{AB}$ and $\overline{CD}$ the following relation between S and H holds:

$$S = -\eta(P^0 - P)/[k_3 \cdot EH_2]. \qquad [10]$$

Substituting [10] into [9], we can evaluate the relaxation oscillation period and find that the period approaches a constant in the limit $S^0 \to \infty$. The numerical calculation is compared for various values of S^0 in the caption of Fig. 1*b*. The temporal course of H is given in that figure and is seen to be a periodic train of short pulses.

IV. Threshold conditions and effect of fluctuations

Many biological phenomena occur which require a minimum non-zero excitatory perturbation to elicit a much larger standard response that follows a temporal development relatively insensitive to any of a large class of excitatory perturbations (23, 24). We find such a phenomenon may be attained in the present system, and we study its behavior in the presence of random fluctuations in the external environment S^0.

A. Threshold and Excitation. In Fig. 2 (upper panel), we see a phase plane diagram indicating the existence of a single steady-state. If the system with stable steady state at O_1 is perturbed to point *a*, below the threshold separatrix, then the system evolves for a relatively long time on a standard trajectory $aABCO_1$, and ends up again at the stable steady state O_1. If, however, the system is initially perturbed to points *b*, or *c* above the separatrix, then the system evolves back to the steady-state in a relatively short time. Recall that little time is spent by the system in the horizontal trajectories. In Fig. 2 (lower panel), we see the time evolution of the system for supra- and subthreshold initial perturbations.

Similar phenomena may be expected when the steady-state is located at O_2, the only difference being that the threshold is

in the opposite direction. If the system is prepared, however, in such a way that it has three steady states, O_1 and O_2 being stable and O_3 unstable (not shown but located between the maximum and minimum on the curve $dH/dt = 0$), then the system when perturbed supra-threshold undergoes an excitation to another steady-state and does not return to the original steady-state.

B. Effect of Fluctuations. If a system under threshold conditions is subject to noise (random perturbations), we expect the behavior of the system to vary as the rms (root mean square) effect of the noise becomes sufficient to perturb the system beyond the threshold. We have studied this phenomenon by subjecting the system to random fluctuations in the external substrate concentration. We do so by changing the substrate concentration S^0 at random times; each change is of gaussian form in time, with amplitude and half-width chosen randomly (by generating random numbers on a computer), subject to a chosen average amplitude and rms width.

The equation [6.2] then remains unchanged (for simplicity we neglect variations in the external H, P, OH) and is taken to obey an equation of the Langevin type,

$$dS/dt = -k_3 \cdot EH_2 \cdot S + h_S(1 - S) + h_S \Delta S^0(t), \quad [11]$$

where the average value $\langle \Delta S^\circ \rangle$ over the ensemble is zero and ΔS° ($= (\Delta S^\circ)/\langle S^\circ \rangle$) must be limited to physically allowed values

$$-1 < \Delta S < \infty. \quad [12]$$

Qualitatively the behavior of the system passes through three regions as the rms noise is increased. If the noise is sufficiently small, then the system is only occasionally brought supra-threshold and the time course of the system (see Fig. 3*a*) is seen to be a sequence of uncorrelated randomly triggered pulses (of which only one is shown). At intermediate values of the noise level, the system does not reside in the vicinity of the steady state very long and is, upon arrival at the steady state, soon perturbed supra-threshold. The resulting behavior (Fig. 3*b*) is quasi-periodic. At large levels of noise the quasi-periodicity is destroyed. As the threshold is decreased the rms noise level needed to attain quasi-periodicity decreases proportionally.

In Fig. 3*a* we see that for low noise level, the system stays around the steady state for most of the time except when a pulse is generated at random times. The region of low H concentration corresponds to the branch $\overline{AB}$ in Fig. 2 (upper panel). Because this branch is steeper than that of $\overline{CD}$ near the steady state, the effect of the noise at low H concentration is suppressed as compared to that at high H concentration. It is also to be noted that the time required to span the branch $\overline{AB}$ for low and intermediate noise levels is quite comparable to that of a single excitation in Fig. 2 (lower panel) whereas for a high noise level the time duration on the branch $\overline{AB}$ (low H concentration) is irregular, since the system is frequently forced between the branches $\overline{AB}$ and $\overline{CD}$.

If the system has two stable steady states quasi-periodic behavior may also be attained.

Fluctuations in a nonlinear threshold system are seen to have the effect of altering the critical values of parameters of the system such as S^0 for which oscillatory behavior may occur. Thus, we found quasi-periodic behavior with imposed noise in a regime where oscillations do not occur according to the deterministic equations, i.e., the equations with $\Delta S = 0$ [11].

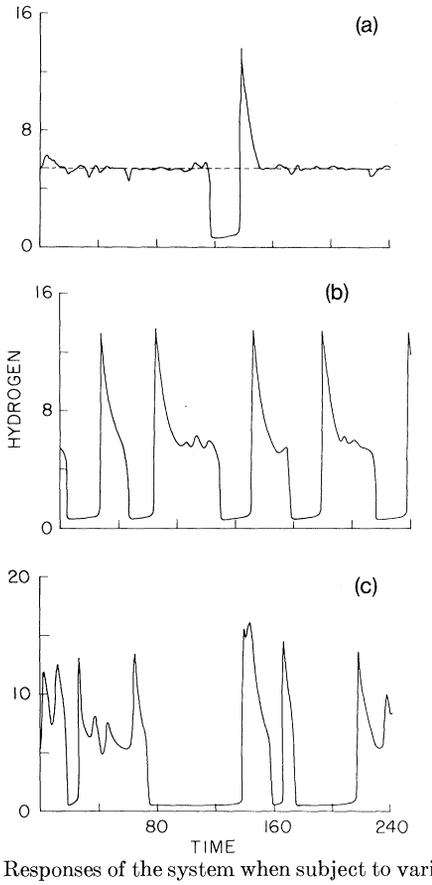

FIG. 3. Responses of the system when subject to various levels of noise, Eq. [11]; hydrogen ion concentration vs. time. Quasi-periodicity for the intermediate noise level is evident. Note the different scale for the high noise level. (*a*) Low noise level, (*b*) Intermediate noise level, (*c*) High noise level.

V. Concluding remarks

The phase plane (S, H) of the system analyzed here bears a striking resemblance to the mathematical model introduced by FitzHugh (12), a modified two-variable van der Pol equation (Bonhoeffer–van der Pol, BVP model). FitzHugh has discussed in length a variety of features of the model: the division of the phase plane "into regions corresponding to the physiological states of nerve fiber (resting, active, refractory, enhanced, depressed, etc.)"; threshold excitation and single pulses; finite trains of successively damped pulses; and infinite trains of pulses. He has compared the two-variable model with projections of the four-variable Hodgkin-Huxley (25) equations and finds the model representative. All the features of BVP model are common to our model.

The variations in behavior found for a system with threshold excitation, on imposition of varying levels of noise, may be conjectured to have applications. If oscillatory behavior is normal for a given biological system, then this state can be achieved by a threshold system at intermediate noise levels. This type of oscillatory behavior is attainable under less stringent concentration conditions than a limit cycle. Furthermore the system has a resting, non-oscillatory, state available at low noise levels.

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