Collapse and Revival of Glycolytic Oscillation

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Glycolysis is the major source of metabolic energy in almost all living cells. A key feature of the glycolytic oscillations is their critical control by substrate injection rate. We show that in the limit of weak noise of the fluctuating substrate injection rate a new instability arises in the dynamics leading to collapse and revival of glycolytic oscillation reminiscent of "bursting" of action potential in nerve cells. The dynamical system in this limit also exhibits an interesting mirror image symmetry between growth and decay of fluctuations of the reaction product.

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Biological oscillators are ubiquitous in natural sciences. Among them, glycolysis serves as one of the most important and prototype examples of limit cycle oscillations [1–3]. This is the major source of metabolic energy in almost all living organisms. In this process, the sugar molecule is converted into the product via a series of enzyme-catalyzed reactions. Over the years, the subject has received considerable attention [1,4–12]. It has now been established that glycolytic oscillations arise [4,5] from complex regulatory properties of allosteric enzymes [11,12], particularly phosphofructokinase (PFK) which itself is activated by adenosine diphosphate, a reaction product [9,10]. A key feature of the glycolytic oscillations is their critical control by substrate injection rate. Both regular and stochastic variations of this parameter have been carried out experimentally [1,12,13] to analyze the nature of nonlinear dynamics of glycolysis. The object of the present paper is to explore a few generic features of this dynamics in the weak noise limit of the fluctuating substrate injection rate. To this end, we consider the large and rare fluctuations of the dynamical variables [14] such as product concentration of the order $\gg \sqrt{D}$, $\sqrt{D}$ being the strength of the injection rate. We show that in the limit $D \to 0$ (i) a new instability arises resulting in collapse and revival of glycolytic oscillations which is reminiscent of bursting of action potential in nerve cells. (ii) Growth of large fluctuations of the dynamical variables away from the stable state in the presence of noise and its return to the stable state along a relaxational path in the absence of noise exhibit a mirror-image symmetry. In the weak noise limit, stochastic processes can be described by appropriate auxiliary Hamiltonian or path integral methods employed recently in several contexts [14,15].

To start with, we consider a simple product activated enzyme reaction model [1,11,12] assuming that the allosteric enzymes consist of multiple identical subunits which undergo conformational transition [11] between more reactive (R) and less reactive (T) states. The substrate S is injected at a rate ν which binds with R and T states of the enzyme and is transformed into the product P. The product is removed at a rate proportional to its concentration and exerts a positive feedback by activating the transition from T to R states. The process is depicted schematically in Fig. 1. The dynamics is described by normalized product concentration γ and substrate concentration α in continuously stirred yeast extracts in terms of the equations [1]

$$d\alpha/dt = \nu - \sigma\phi(\alpha, \gamma), \tag{1}$$

$$d\gamma/dt = q\sigma\phi(\alpha, \gamma) - k_s\gamma, \tag{2}$$

$$\phi(\alpha, \gamma) = \frac{\alpha e(1 + \alpha e)^{n-1}(1 + \gamma)^n + L\theta\alpha ce'(1 + \alpha ce')^{n-1}}{L(1 + \alpha ce')^n + (1 + \alpha e)^n(1 + \gamma)^n}. \tag{3}$$

Here the normalized substrate and product concentrations are defined by $\alpha = [S]/K_R$ and $\gamma = [P]/K_P$, respectively. $q = K_R/K_P$ denotes the ratio of the dissociation constants of the reactant and product with the enzyme in the R state. σ is the maximum capacity of the enzyme to transform the substrate into the product. k_s refers to the first order rate constant for removal of the product. L is the allosteric constant of the enzyme and is given by $[T]/[R] \gg 1$ without ligand. $c = k_R/k_T$ is the

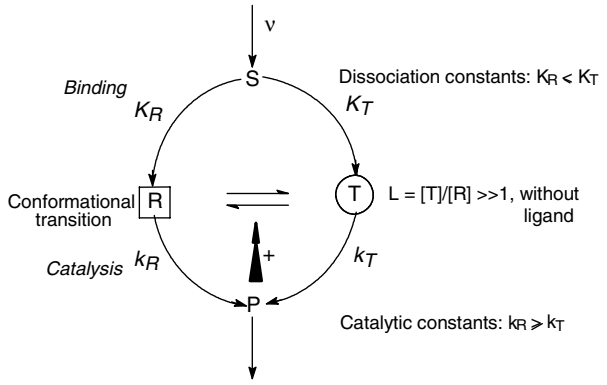

FIG. 1. Goldbeter scheme of an autocatalytic enzyme reaction in an allosteric model for glycolytic oscillation.

nonexclusive binding constant of the substrate. θ is the ratio of the catalytic activity of enzyme in T and R states. e and e' are two constants determined as $e = 1/(1+\epsilon)$ and $e' = 1/(1+\epsilon')$, where $\epsilon = k/d$ and $\epsilon' = k'/d'$. k' and k are the respective rate constants for the steps when the enzyme in T and R states converts a substrate molecule to the product. d' and d are the respective rate constants for the steps where detachment of a substrate molecule occurs from an enzyme in the T and the R state, respectively.

Following Goldbeter [1], we consider the simplest case when the enzyme is a dimer and the substrate binds exclusively to the R state of the enzyme; i.e., $\theta = 0$ and $e' = 1$. This captures the essential features of positive cooperativity. $\phi(\alpha, \beta)$ then assumes a simple form with $n = 2$. The limit cycle oscillations of the allosteric model originate from the regulatory properties of PFK, and it has been possible to vary experimentally the substrate injection rate in constant, periodic, and random manner within physiologically accepted values of the experimental parameters [1,12,13,16] relevant for yeast cells. We therefore consider a small but rapid fluctuation of the substrate injection rate $\nu(t)$ and rewrite (1) and (2) as

$$d\alpha/dt = \eta(t) + f(\alpha, \gamma), \tag{4}$$

$$d\gamma/dt = g(\alpha, \gamma), \tag{5}$$

where the dynamical system is driven by the weak white noise $\nu(t)$ [$= \nu_0 + \eta(t)$] whose mean and variance can be expressed as

$$\langle \nu(t) \rangle = \nu_0 \qquad \langle \eta(t)\eta(t-\tau) \rangle = D\delta(\tau), \tag{6}$$

such that $\sqrt{D} \ll 1$ and D can be considered as the smallness parameter for the present problem of singular perturbation theory that follows. Here $f(\alpha, \gamma)$ and $g(\alpha, \gamma)$ are given by $f(\alpha, \gamma) = \nu_0 - \sigma\phi$ and $g(\alpha, \gamma) = q\sigma\phi - k_s\gamma$. The experimental justification for ascribing the nature of fluctuation of the substrate injection rate as above lies in the fact that the injection rate can be varied stochastically rapidly in the time scale over which glycolytic oscillation takes place [13]. The Fokker-Planck equation for probability distribution function $P(\alpha, \gamma, t)$ corresponding to Langevin description (4)–(6) can be written down as

$$\frac{\partial P}{\partial t} = -\frac{\partial(fP)}{\partial \alpha} - \frac{\partial(gP)}{\partial \gamma} + D\frac{\partial^2 P}{\partial \alpha^2}. \tag{7}$$

In the weak noise limit $Lt_{D\to 0}$, $P(\alpha, \gamma, t)$ can be described [14,15] by a WKB-type approximation of the Fokker-Planck equation (7) of the form $P(\alpha, \gamma, t) = P_0(\alpha, \gamma, t)\exp[s(\alpha, \gamma, t)/D]$. Here P_0 is a prefactor and $s(\alpha, \gamma, t)$ is the classical action satisfying Hamilton-Jacobi equation which can be solved by auxiliary Hamilton's equations of motion,

$$d\alpha/dt = \nu_0 - \sigma\phi - 2p, \tag{8}$$

$$d\gamma/dt = q\sigma\phi - k_s\gamma, \tag{9}$$

$$dx/dt = \sigma\phi_\gamma p - q\sigma\phi_\gamma x + k_s x, \tag{10}$$

$$dp/dt = \sigma\phi_\alpha p - q\sigma\phi_\alpha x, \tag{11}$$

where $\phi(\alpha, \gamma)$ is given by Eq. (3) for $n = 2$, and ϕ_α and ϕ_γ are the derivatives of ϕ with respect to α and γ, respectively. The auxiliary Hamiltonian is given by

$$H_{\text{aux}}(\alpha, x, \gamma, p) = -f(\alpha, \gamma)p - g(\alpha, \gamma)x + p^2, \tag{12}$$

where $p = \partial s/\partial\alpha$ and $x = \partial s/\partial\gamma$.

The origin of auxiliary dynamical variables x and p is the fluctuation of the substrate injection rate. The introduction of these variables implies addition of a new degree of freedom in the dynamics originally described by α and γ. Since x, p owe their existence in the limit $D \to 0$, we must look for the influence of vanishing weak noise in the injection rate in the limit $x \to 0$ and $p \to 0$, so that the auxiliary Hamiltonian H_{aux} tends to zero. In what follows, we show that the vanishing Hamiltonian method captures the essential features of some generic effects of weak noise in the dynamics of glycolytic oscillations.

The steady state solutions $(x_0, p_0, \alpha_0, \gamma_0)$ of the extended dynamical system (8)–(11) can be obtained as $x_0 = 0$, $p_0 = 0$ while γ_0 and α_0 remain the same as they are in the unperturbed system, i.e., $\gamma_0 = q\nu_0/k_s$, and α_0 is determined by the solution of the equation $\nu_0 = \sigma\phi(\alpha_0, \gamma_0)$. Linearizing the kinetic equations (8)–(11) around the fixed point, the condition for instability can be determined by the following characteristic equation eigenvalues (w) of the stability matrix:

$$w^2 + w(AC + k_s - qBC) + ACk_s = 0, \tag{13}$$

$$w^2 + w(qBC - k_s - AC) + ACk_s = 0, \tag{14}$$

where A, B, and C are given by

$$\begin{aligned} A = {} & eL(1+\alpha c)^2(1+2\alpha e)(1+\gamma)^2 \\ & + e(1+\alpha e)^2(1+\gamma)^4 \\ & - 2ec\alpha L(1+\alpha e)(1+\alpha c)(1+\gamma)^2, \end{aligned} \tag{15}$$

$$B = 2\alpha eL(1+\alpha e)(1+\gamma)(1+\alpha c)^2, \tag{16}$$

$$C = \frac{\sigma}{[L(1+\alpha c)^2 + (1+\alpha e)^2(1+\gamma)^2]^2}. \tag{17}$$

The condition for instability corresponding to Eq. (13) is the same [1] as that for the unperturbed system, i.e., $AC - qBC + k_s < 0$, since by virtue of experimental condition AC is always greater than zero. From Eq. (14), we see that one of the roots of this equation is always positive regardless of the sign of $-qBC + AC + k_s$. These two conditions therefore imply that the steady state of the extended dynamical system is always linearly unstable. The passage through the critical point of instability (i.e., Hopf bifurcation) corresponding to Eq. (13) results in sustained limit cycle oscillations. To explore further, we resort to numerical simulation of Eqs. (8)–(11).

The basis of our simulation essentially rests on the phase plane analysis of the unperturbed dynamics (Fig. 2). The effect of substrate injection rate and the nature of dynamics depend critically on the intersection of the product nullcline ($d\gamma/dt = 0$) with the substrate nullcline ($d\alpha/dt = 0$). When the steady state is located at the intersection of the two nullclines, lying at the negative slope of the product nullcline (for $\nu_0 = 0.4$, for example) the system admits of limit cycle oscillations surrounding the steady state. Such a situation is depicted in Fig. 3(a) in the α-γ plane for the parameter set [1] $n = 2$, $\nu_0 = 0.4\ s^{-1}$, $\sigma = 10^3\ s^{-1}$, $k_s = 0.1\ s^{-1}$, $q = 1.0$, $L = 7.5 \times 10^6$, $c = 0.01$, and $e = 0.9091$, for the unperturbed system. The corresponding variation of product concentration γ with time is shown in the inset of Fig. 3(a).

The noise degree of freedom described by auxiliary variables x, p is now switched on. To this end, Eqs. (8)–(11) are numerically integrated with vanishingly small initial values of x and p as $x = 1 \times 10^{-6}$ and $p = 1 \times 10^{-6}$ for the same parameter set. The result is shown in Fig. 3(b). With the passage of time, the limit cycle phase curve moves towards an attractor, and stays there for some time, only to return to the limit cycle curve, and so on. The process continues asymptotically. In the inset of Fig. 3(b), we plot the variation of γ as a function of time. It is apparent that after a lapse of considerable periods the oscillations collapse so that the system remains in the quiescent state for some time followed by a revival of oscillations. This collapse and revival of glycolytic oscillations is similar to bursting behavior in many neuron cells [17], where one encounters relatively quiescent hyperpolarized periods alternating with periods in which a series of burst of action potential occurs. The bursting behavior has also been observed in oscillatory chemical reactions in a continuously stirred tank reactor [17,18]. A plausible origin of collapse and revival of glycolytic oscillations can be understood in terms of mixed mode oscillations of the system (α, γ) and noise (x, p) degrees of freedom and a slow manifold picture employed earlier in several contexts [17–21]. This essentially implies that, in the course of rapid passage between the left and the right sheets of the S-shaped manifold (Fig. 2), the system stays in the right sheet only to move out slowly from it, centering around the steady state for some time until it reaches the edge of the right sheet to jump again to the left sheet. An important point regarding the phenomenon is that it can be observed for the set of initial conditions of α, γ located in the S region of the phase space enclosed by a limit cycle as shown in Fig. 3(c). The initial conditions other than this (i.e., located in the U region) lead to divergence of oscillations.

The nonlinear dynamics of glycolysis exhibits another interesting feature in the weak noise limit of the substrate injection rate. To demonstrate the basic idea, we set the parameter values [1] governing the dynamics of the system (8)–(11) as $n = 2$, $\nu_0 = 0.7$, $q = 1.0$, $k_s = 0.1\ s^{-1}$, $L = 7.5 \times 10^6$, $\sigma = 1000$, $c = 0.01$, and $e = 0.9091$. The stable fixed point (say X_0) of the unperturbed system in this case corresponds to $\alpha_0 = 10.474$ and $\gamma_0 = 6.999$. With the introduction of noise degree of freedom,

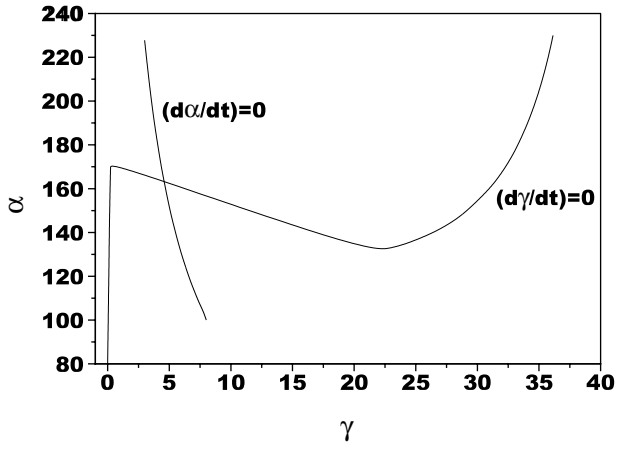

FIG. 2. Product ($d\gamma/dt = 0$) and substrate ($d\alpha/dt = 0$) nullclines for glycolytic oscillation for the parameter values mentioned in the text. The substrate nullcline is drawn for $\nu_0 = 0.4\ s^{-1}$.

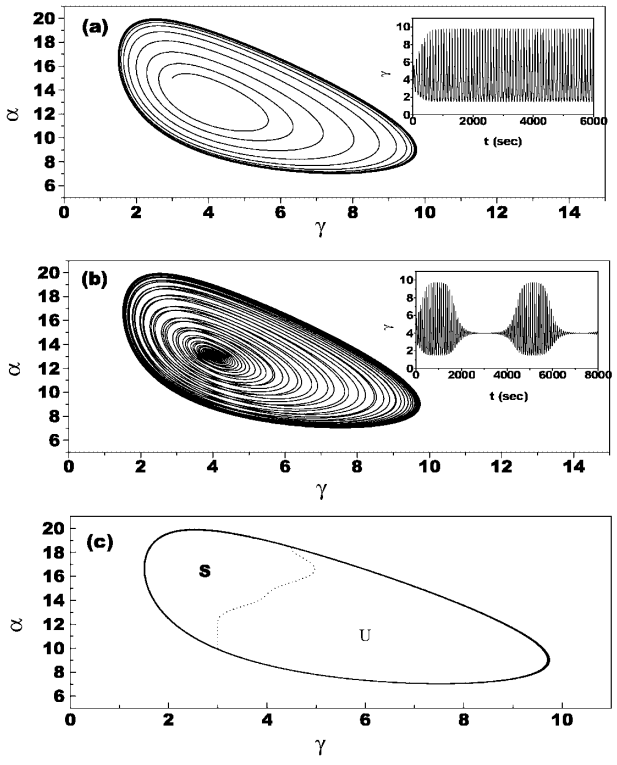

FIG. 3. (a) Limit cycle curve (α vs γ plot) for the parameter set mentioned in the text in the absence of noise [inset: sustained oscillation of the product (γ) concentration as a function of time t (in sec) in the absence of noise]. (b) Same as in (a) but in the presence of noise [inset: same as in (a) but in the presence of noise]. α and γ are dimensionless quantities. (c) Region "S" within the limit cycle designates the zone of the initial conditions that lead to collapse and revival phenomena of glycolytic oscillation.

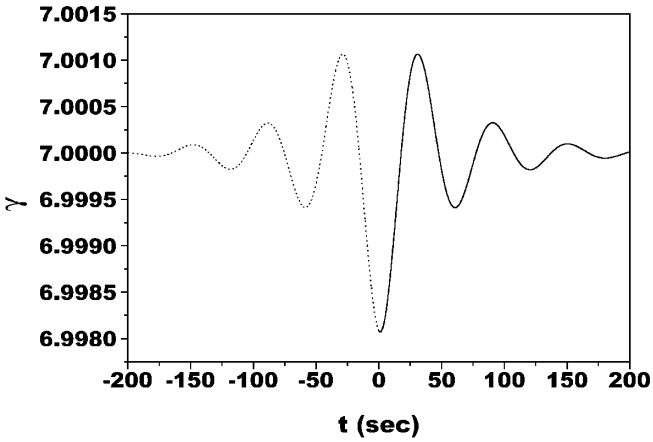

FIG. 4. The mirror image symmetry between the path (dotted line) signifying the fluctuation of dimensionless γ as a function of time t (in sec) from the steady state (in the presence of noise) and relaxational path (continuous line) towards the steady state (in the absence of noise).

the trajectory moves away from X_0 to reach a preassigned state (say X_f) of large fluctuation (compared to strength of noise x, $p \approx 10^{-6}$) at $t = 0$ as shown in Fig. 4. The fluctuational path from X_0 to X_f can be identified as the optimal path along which the system moves with an overwhelming large probability [14]. If the system is now allowed to follow a relaxational path from X_f in the absence of noise variables to reach the steady state X_0, one observes a mirror image symmetry between the growth of fluctuation from X_0 to X_f and its decay from X_f to X_0. Although such a symmetry has been demonstrated experimentally as a proof of the principle of detailed balance in analogue electronic circuits [14], where the dynamical system in question is closed thermodynamically, it is somewhat counterintuitive in the present context since the dynamical system describing glycolysis is thermodynamically open because of the absence of any relation between external noise and dissipation. We therefore believe that the observed symmetry in the present case is different from the former [14] and originates from an interplay of nonlinearity and dissipation in the system such that the energy is injected in one region and is extracted in another region of phase space. The existence of a limit cycle signifies a common boundary between them and a delicate balance (not to be confused with detailed balance) between the rate of excitation or supply of energy and the loss due to dissipation ensuring a strict periodicity and recovery from weak noise imposed on the system.

In summary, we have shown that nonlinear dynamics of glycolysis in the weak noise limit of the substrate injection rate admits of an interesting phenomenon of collapse and revival of glycolytic oscillation similar to bursting of action potential in nerve cells and a mirror image symmetry of growth and decay of large fluctuations in the dynamical system. Since the features are generic in the singular perturbative limit, we believe that the present study can be extended to other models in biology where limit cycles play an important role in situations far from equilibrium.

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