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ON THE ROLE OF ENZYME COOPERATIVITY IN METABOLIC OSCILLATIONS: ANALYSIS OF THE HILL COEFFICIENT IN A MODEL FOR GLYCOLYTIC PERIODICITIES

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The role of enzyme cooperativity in the mechanism of metabolic oscillations is analyzed in a concerted allosteric model for the phosphofructokinase reaction. This model of a dimer enzyme activated by the reaction product accounts quantitatively for glycolytic periodicities observed in yeast and muscle. The Hill coefficient characteristic of enzyme–substrate interactions is determined in the model, both at the steady state and in the course of sustained oscillations. Positive cooperativity is a prerequisite for periodic behavior. A necessary condition for oscillation in a dimer K system is a Hill coefficient larger than 1.6 at the unstable stationary state. The analysis suggests that positive as well as negative effectors of phosphofructokinase inhibit glycolytic oscillations by inducing a decrease in enzyme cooperativity. The results are discussed with respect to glycolytic and other metabolic periodicities.

1. Introduction

The physiological significance of metabolic oscillations is progressively coming to light, the best example being the role of periodic cAMP pulses in the differentiation of cellular slime molds [1, 2; see ref. 3 for a review]. Glycolytic oscillations are the prototype of periodicity in a metabolic pathway [3]. These oscillations, observed in yeast and muscle [4–6], result from the cooperative and regulatory properties of phosphofructokinase (PFK) *. An allosteric model has been developed for the PFK reaction [7, 8], in the frame of the concerted transition theory of Monod et al. [9]. The model yields agreement with the oscillatory dynamics of the glycolytic system, and allows a detailed analysis of the molecular mechanism of oscillations in this pathway [3, 7, 8, 10].

The positive feedback exerted on PFK by a reaction product and the far from equilibrium operation of this enzyme play an essential role in the origin of glycolytic periodicities [3–8, 10]. Experimental results on the periodic on-off performance of PFK suggest that the allosteric nature of the enzyme is of equal importance for oscillatory behavior [4]. The purpose of the present study is to determine the role of enzyme cooperativity in the onset of metabolic oscillations. To this end, the Hill coefficient characteristic of enzyme–substrate interactions is analyzed in the allosteric model for PFK. The motivation for this analysis is twofold: the Hill coefficient is the most widely used measure of cooperativity in allosteric enzymes; furthermore, its analytical expression in the concerted model is known [11–13].

It is well established that sustained periodicities in chemical systems occur around a nonequilibrium unstable stationary state [14, 15]. Here we show that sustained oscillations in the PFK model are associated with large values of the Hill coefficient at the unstable steady state. These values are close to the number of protomers constituting the enzyme. Moreover, the Hill coefficient remains near maximum during oscillation.

The analysis of the Hill coefficient provides an explanation for the fact that positive as well as negative effectors of PFK inhibit glycolytic oscillations. The results support the conclusion that enzyme cooperativity plays a primary role in the mechanism of glycolytic and other metabolic periodicities.

* Abbreviations: PFK, phosphofructokinase (E.C. 2.7.1.11); F6P, fructose-6-phosphate; FDP, fructose-1,6-diphosphate.

2. Allosteric model for glycolytic oscillations

The model developed for the PFK reaction in the frame of the concerted transition theory [9] is that of an open K–V system in which the product is a positive effector of the dimer enzyme (see ref. [7] and [8] for a detailed presentation). When the effect of diffusion remains negligible, as in continuously stirred extracts of yeast or muscle [4–6], the time evolution of substrate and product concentrations is described by the following kinetic equations [7, 8]:

$$\frac{d\alpha}{dt} = \sigma_I - \sigma_M \Phi , \qquad \frac{d\gamma}{dt} = \sigma_M \Phi - k_s \gamma , \tag{1}$$

with

$$\Phi = (v/V_M) = \frac{\alpha e(1+\alpha e)(1+\gamma)^2 + L\theta\alpha c e'(1+\alpha c e')}{L(1+\alpha c e')^2 + (1+\gamma)^2(1+\alpha e)^2} \tag{2}$$

Here, α and γ denote respectively the concentration of substrate (ATP or F6P) and product (ADP or FDP) of the PFK reaction, divided by the dissociation constant K_R of enzyme complexes in the R state. The allosteric constant L denotes the ratio of enzyme forms in the T and R states in the absence of ligands; the nonexclusive binding coefficient c, equal to the ratio K_R/K_T, reflects the differential affinity of the substrate for these states; σ_I and σ_M denote the substrate input rate and the maximum enzyme reaction rate, divided by K_R; k_s is the rate constant for the product sink; θ represents the ratio of catalytic constants of the T and R conformations. Finally, $e = (1+\epsilon)^{-1}$ and $e' = (1+\epsilon')^{-1}$, where ϵ and ϵ' are relative catalytic constants of the T and R states. Further details on the definition of these parameters are given in refs. [7, 8, 10].

A stability analysis of the stationary state (α_0, γ_0) admitted by eq. (1) for $(d\alpha/dt) = (d\gamma/dt) = 0$ yields the conditions in which the system undergoes sustained oscillations of the limit cycle type [7, 8]. The oscillatory behavior of the model is in agreement with experimental findings in glycolyzing yeast extracts as to the oscillatory range of substrate injection rates, periodic variation of enzyme activity, control of period and amplitude by the substrate input, phase shift by the product, entrainment by a periodic source of substrate, and stability in the presence of random variations of the substrate input [3, 7, 8, 10]. It follows that the oscillatory dynamics of the glycolytic system can satisfactorily be described by the allosteric model for the PFK reaction.

3. Enzyme cooperativity and sustained oscillations

The domain of sustained oscillations in the PFK model usually corresponds to large values of the allosteric constant L [7, 8]. These values, of the order of 10^6, suggest that enzyme cooperativity plays a primary role in the onset of periodic behavior. This is confirmed by a quantitative analysis of the Hill coefficient at the stationary state.

In enzymatic systems, the Hill coefficient is defined as the slope of a Hill plot established either with respect to the equilibrium saturation function of the enzyme, or with respect to the reaction rate. For each definition, an analytical expression of the Hill coefficient has been obtained in the concerted model for allosteric enzymes [11–13]. In perfect K systems, in which the T and R states have the same catalytic activity ($\theta = 1$), the analytical expression of the Hill coefficient remains similar for binding [11] or kinetic data [12, 13]. The kinetic Hill coefficient related to the substrate is then given, in the PFK model, by the relation [13]:

$$n_H = 1 + \alpha L'(e - ce')^2 / \{[(1+\alpha e) + L'(1+\alpha c e')] \times [e(1+\alpha e) + L' c e'(1+\alpha c e')]\} , \tag{3}$$

where the apparent allosteric constant L' is defined [9] as

$$L' = L/(1+\gamma)^2 . \tag{4}$$

At equilibrium, $e = e' = 1$; eq. (3) then yields the expression of the Hill coefficient defined for binding [11–13].

The value of the Hill coefficient at the stationary state is obtained by inserting the steady-state concentrations α_0, γ_0 (see the appendix) in relations (3) and (4).

As shown in previous studies of the concerted model [16], the Hill coefficient in a perfect K system goes to unity when the substrate has the same affinity for the T and R states ($c = 1$), and tends to its maximum value, equal to the number of protomers constituting the enzyme, when the substrate binds exclusively to the R state ($c = 0$). A plot of the Hill coefficient at the

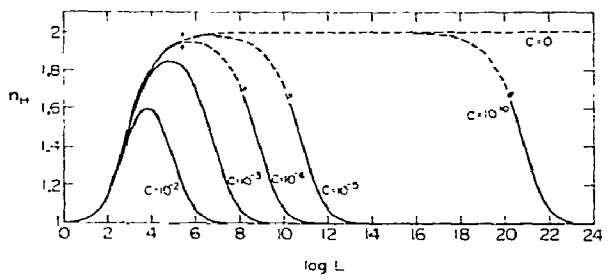

Fig. 1. Hill coefficient at the stationary state and oscillatory domain, as a function of the allosteric constant L and of the nonexclusive binding coefficient c. The Hill coefficient n_H is calculated according to eq. (3), at the steady state given by eq. (A.1) and (A.3) in the appendix. On each curve, the segment of dashed line between arrows indicates periodic behavior, of the limit cycle type, around an unstable stationary state; the stability properties of the steady state are determined by normal mode analysis [7,8]. The curves are established on an IBM 370-165 computer, for the following set of parameter values corresponding to experimental data in oscillating yeast extracts [3,4,7,8,10]: $c = c' = 10^{-3}$, $\sigma_I = 0.7\ s^{-1}$, $k_S = 0.1\ s^{-1}$, $\sigma_M = 4\ s^{-1}$; the dimer enzyme considered is a perfect K system ($\theta = 1$). Similar curves are obtained when the Hill coefficient is plotted as a function of the apparent allosteric constant L'.

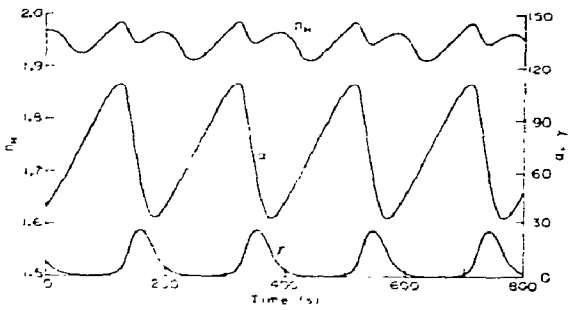

Fig. 2. Hill coefficient and metabolite concentrations in the course of sustained oscillations. The periodic variation of the normalized substrate (α) and product (γ) concentrations is obtained by integration of eq. (1) on an IBM 370-165 computer, for $\epsilon = \epsilon' = 0.1$, $L = 10^6$, $c = 10^{-5}$ (other parameters as in fig. 1); initial conditions are $\alpha = 40$, $\gamma = 8$. The corresponding variation of the Hill coefficient n_H is determined according to eq. (3). Actual metabolite concentrations are obtained by multiplying α and γ by the dissociation constant $K_R = 5 \times 10^{-2}$ mM [7,8].

stationary state as a function of the nonexclusive binding coefficient of the substrate shows, in the PFK model, that sustained oscillations occur below a critical value of c when the allosteric constant is sufficiently large [8].

The relationship between the steady-state value of the Hill coefficient and the allosteric constant is illustrated in fig. 1, for different values of the nonexclusive binding coefficient. Bell-shaped curves thus obtained resemble those that yield the maximum Hill coefficient for binding as a function of the apparent allosteric constant [16]. On each curve, the segment of dashed line indicates the domain of sustained oscillations. For a given value of c, there is a finite domain of L values yielding oscillation, except when the substrate binds to the R state of the enzyme exclusively ($c = 0$).

The oscillatory domain of n_H values presents a certain asymmetry with respect to the allosteric constant. This asymmetry results from that the substrate concentration at the steady state has to accumulate above a critical level for instability to occur; such an accumulation takes place for values of L larger than 10^5 [8].

It can be seen that only large values of the Hill coefficient, close to the maximum value, are associated with instability and periodic behavior. A numerical study of the model over a wide range of parameter values indicates that a necessary prerequisite for instability of the steady state in a dimer K system is a Hill coefficient larger than 1.6 in this state.

In the course of oscillation, the Hill coefficient varies periodically but remains in the neighborhood of its maximum value (fig. 2). The double periodicity exhibited in the time variation of n_H reflects the dependence of the Hill coefficient on both substrate and product concentrations, which do not oscillate in phase, as shown in fig. 2.

4. Discussion

The study of the Hill coefficient at the stationary state in the allosteric model for the PFK reaction illustrates well the essential role of enzyme cooperativity in the mechanism of glycolytic oscillations. This study indicates that positive cooperativity of enzyme–substrate interactions is a necessary prerequisite for sustained oscillatory behavior.

The above results can be related to some experimenta

observations. The maximum number of cycles in glycolyzing yeast extracts is obtained around pH 6.5 [17]. Similar results were reported for muscle extracts [6]. These observations suggested [3,8,10] a role for enzyme cooperativity in the mechanism of glycolytic instability, since the PFK from many sources behaves as a Michaelian enzyme near pH 8, and becomes allosteric at acidic pH [18]. Available data on the pH dependence of PFK cooperativity in yeast [19] would not seem to support such straightforward interpretation of the effect of pH on oscillation; this effect might in part arise from the pH dependence of enzyme activity [19]. The domain of sustained oscillations in yeast nevertheless corresponds to the region of maximum cooperativity in the saturation curve of PFK by its substrate F6P [4].

Positive effectors of PFK, such as ammonium ions [4,20], as well as negative effectors, such as citrate [6], inhibit glycolytic oscillations. Similar results are obtained with indirect effectors of PFK [20,21]. The present analysis suggests that these effectors all interfere with the oscillatory mechanism by lowering PFK cooperativity. Positive and negative effectors bring about such effect by inducing a decrease and an increase in the apparent allosteric constant, respectively. Both types of action may result in lowering the Hill coefficient below some critical value, as indicated in fig. 1. Addition of an inhibitor should not hinder oscillations in the case of exclusive or quasi-exclusive binding of substrate to the R state, as shown by the curves corresponding to $c = 0$ and $c = 10^{-10}$. In such a situation, an increase in L' can at most induce a lengthening of the period [8].

The physical interpretation of the above results can be obtained from a discussion of the kinetic equations. Such a discussion throws light on the interplay of positive feedback and cooperativity in the mechanism of instability in glycolysis. The term $(1 + \gamma)^2$ in eq. (2) reflects the activation of the enzyme by the reaction product. This autocatalysis destabilizes the stationary state [7,8]. As long as the allosteric constant L remains small, the effect of positive feedback is negligible, as most of the enzyme is already present in the R state, which has the largest affinity for the substrate, in the absence of ligands. Then the ratio (v/V_M) in eq. (2) reduces to Michaelian form; oscillations do not occur in these conditions. Autocatalysis becomes preponderant only when the cooperativity of allosteric interactions is large, i.e., for large values of L and small values of c. It should be noted that instability can occur for lower values of L in a K–V system when the substrate binds significantly to the less active T state, since such binding then enhances the effect of autocatalysis [7,8].

The present calculations have been carried out on a dimer enzyme model. Dimers are of special interest for the determination of the role of cooperativity in the mechanism of sustained oscillations, since they represent the minimal situation in which cooperative interactions can take place in multisubunit enzymes. The results on the role of cooperativity in the onset of oscillations in a dimer system would likely extend to enzymes formed of a larger number of protomers. Indeed a larger subunit number does not change significantly the oscillatory properties of the system [22], nor does it change the discussion on the relative magnitude of the allosteric constant and positive feedback in the kinetic equations.

Cellular slime molds further exemplify the role played by the cooperativity of allosteric enzymes in the onset of metabolic oscillations. In *Dictyostelium discoideum*, sustained oscillations of cAMP control amoeboid differentiation [1,2,23]. A mechanism for the periodic synthesis of cAMP in *D. discoideum* has been proposed, based on the regulatory properties of adenyl cyclase [24]. The cooperative kinetics of this enzyme is of primary importance in the mechanism of oscillation since the analysis of the model indicates, as in glycolysis, that large values of the allosteric constant are associated with instability of the stationary state.

Models for oscillations in a sequence of enzymatic reactions controlled by end-product inhibition also show that cooperativity of the feedback process is a necessary prerequisite for periodic behavior [25]. Similar conclusions apply to periodicities that originate from genetic regulation [3,25] and from the cooperativity of transport processes in membranes [26]. These results, and those obtained for the PFK model, support the view [27] that cooperative allosteric transitions at the genetic, enzyme, or membrane level might form an essential part of the instability mechanism in many biochemical oscillations.

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Appendix. Metabolite concentrations at the steady state

At the steady state, $(d\alpha/dt) = (d\gamma/dt) = 0$, and the normalized product concentration is

$$\gamma_0 = \sigma_1/k_s . \qquad (A.1)$$

Eq. (1) further yields the relation

$$\alpha_0^2[(\sigma_1 - \sigma_M\theta)L(ce')^2 + (\sigma_1 - \sigma_M)(1+\gamma_0)^2e^2] + \alpha_0[(2\sigma_1 - \sigma_M)e(1+\gamma_0)^2 + (2\sigma_1 - \sigma_M\theta)Lce'] + \sigma_1[L + (1+\gamma_0)^2] = 0 . \qquad (A.2)$$

Denoting by m_1, m_2, and m_3 the coefficients of α_0^2, α_0, and the independent term in this polynomial, we obtain the expression for the normalized substrate concentration at the steady state:

$$\alpha_0 = -[m_2 + (m_2^2 - 4m_1m_3)^{1/2}]/2m_1 . \qquad (A.3)$$

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