MODULATION OF THE ADENYLATE ENERGY CHARGE BY SUSTAINED METABOLIC OSCILLATIONS

Albert GOLDBETER
Laboratory of Membranes and Bioregulation, Weizmann Institute of Science
Rehovot, Israel

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1. Introduction

In a number of cases, periodic phenomena in metabolic pathways [1] involve adenine nucleotides, the best known example being glycolysis [1–6]. The physiological significance of these periodicities linked to the energy state of the cell [6] can be investigated in a theoretical model [7] yielding quantitative agreement with most experiments on glycolytic oscillations. A recent analysis of this model [8] indicates that the coupling between diffusion and oscillatory behavior can give rise to propagating concentration waves at the supracellular level.

In an attempt to gain insight into the role of oscillations within the cell, I have considered here the glycolytic model in the homogeneous case and determined the effect of sustained oscillations on the adenylate energy charge (AEC). This parameter, defined by Atkinson [9] as the mole fraction of ATP plus half the mole fraction of ADP, varies between 0 and 1:

$$\mathrm{AEC} = \frac{[\mathrm{ATP}] + [\mathrm{ADP}]/2}{[\mathrm{ATP}] + [\mathrm{ADP}] + [\mathrm{AMP}]} \qquad (1)$$

In vitro studies have shown that the activity of a number of enzymes depends on the energy charge according to their physiological role: enzymes which belong to energy-yielding pathways are activated at low adenylate charge and inhibited when the energy charge approaches unity, whereas the inverse is true for enzymes from ATP-utilizing pathways [9,10]. The two types of normalized activity curves intersect in the region of steepest variation, around the charge 0.8 which corresponds to the conditions for maximum control of cell metabolism. Pointing to a regulatory role of the charge in vivo is a recent review [11] indicating that the AEC value in a number of tissues and organisms is maintained in the range 0.75–0.9.

In the following, it is suggested that sustained oscillations involving adenine nucleotides may induce an alternating operation of metabolic pathways through modulation of the energy charge.

2. The model

Glycolytic periodicities observed in yeast and muscle extracts as well as in single cells and cell populations of yeast originate from the activation of the allosteric enzyme phosphofructokinase by one of the reaction products [1–6]. Recently, Lefever and I analyzed a model for this reaction [7] in the frame of the concerted transition theory proposed by Monod et al. [12]. The model considered is that of an open K-V system in which the product is a positive effector of the dimer enzyme (see fig. 1). In the homogeneous case where diffusion is neglected, the system is described by the following evolution equations for the metabolite concentrations [7]:

$$\frac{d\alpha}{dt} = \sigma_1 - \sigma_M \Phi \qquad (2a)$$

$$\frac{d\gamma}{dt} = \sigma_M \Phi - k_s \gamma \qquad (2b)$$

where:

$$\Phi = \frac{(\frac{\alpha}{\epsilon+1})(1+\frac{\alpha}{\epsilon+1})(1+\gamma)^2 + L\theta(\frac{\alpha c}{\epsilon'+1})(1+\frac{\alpha c}{\epsilon'+1})}{L(1+\frac{\alpha c}{\epsilon'+1})^2 + (1+\gamma)^2(1+\frac{\alpha}{\epsilon+1})^2} \quad (2c)$$

Furthermore α and γ are, respectively, the normalized concentrations of the substrate S (ATP) and product P (ADP) of the enzyme reaction:

$$\alpha = \frac{[S]}{K_{R(S)}}, \quad \gamma = \frac{[P]}{K_{R(P)}} \quad (3)$$

with

$$K_{R(S)} = K_{R(P)} = d/a \quad (4)$$

The other parameters in eqs. (2a–c) were defined as follows (see fig. 1):

$$\sigma_1 = \frac{V_1}{K_{R(S)}}, \quad \sigma_M = \frac{V_{max}}{K_{R(S)}}, \quad \theta = k'/k, \epsilon = k/d,$$

$\epsilon' = k'/d'$, $K_{T(S)} = d'/a'$ and $c = K_{R(S)} / K_{T(S)}$;
$L = k_1/k_2$ is the allosteric constant of the enzyme and V_{max}, the maximum enzyme reaction rate.

The analysis of equations (2a, b) [7] yields the conditions under which the system undergoes sustained oscillations corresponding to a limit cycle around a nonequilibrium unstable stationary state, i.e., a temporal *dissipative structure* [13] with unique amplitude and frequency. The limit cycle behavior of the model matches the oscillations observed in yeast cells and extracts as to the period, amplitude, enzyme activity, phase-shift by the product and oscillatory range of substrate injection rates [7,14,15].

Since the substrate and the product considered in the model for phosphofructokinase are, respectively, ATP and ADP, the energy charge in the oscillating system can readily be computed. Let us note that the autocatalytic mechanism of glycolytic oscillations can be understood in a simple way with respect to this parameter. Phosphofructokinase utilizes ATP as a substrate and belongs at the same time to an ATP-regenerating system, glycolysis. The enzyme is regulated according to the latter function [10]. It is thus activated at low energy charge, i.e., by the product ADP and AMP.

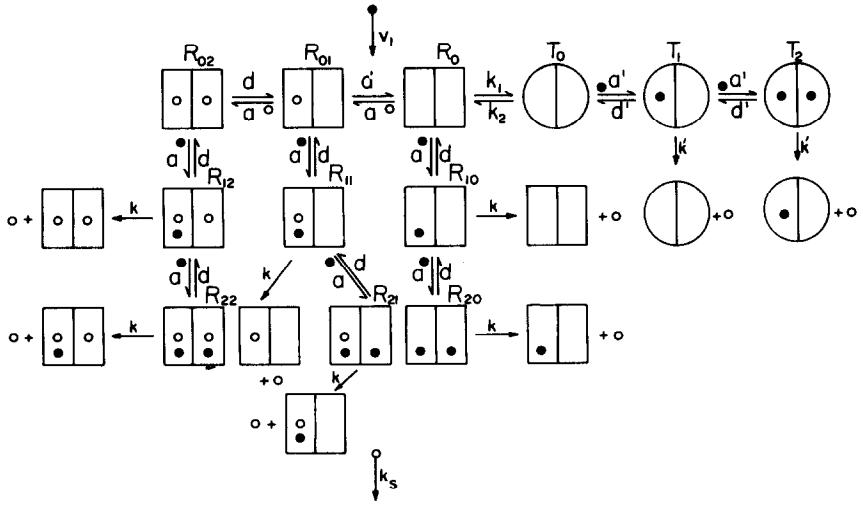

Fig. 1. Model of an allosteric dimer enzyme activated by the reaction product. The substrate (•) is injected at a constant rate; the product (○) binds exclusively to the R state of the enzyme ($\theta, c \leq 1$; see text) and is removed in a monomolecular step.

3. Periodic variation of the energy charge

The adenylate energy charge for the system described by equations (2a, b) is given by:

$$\frac{\alpha + \gamma/2}{\alpha + \gamma} \qquad (5)$$

The denominator of this fraction does not include AMP, the latter not being considered in the model (see Discussion below).

In the narrow oscillatory range of substrate injection rates, the value of the energy charge at the unstable steady state lies between 0.8 and 1 (fig. 2), in agreement with the values generally found in vivo [11].

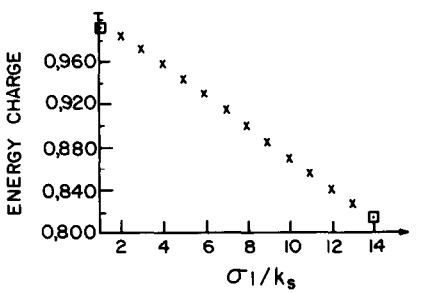

Fig. 2. Energy charge at the unstable stationary state. The squares at the extremities of the oscillatory domain refer to stable steady states. The curve is established according to eq. (5) as a function of the substrate injection rate σ_1 for $L = 5 \times 10^6$, $k_S = 0.1$ sec^{-1}, $\sigma_M = 10^2$ sec^{-1}, $K_{R(S)} = K_{R(P)} = 5 \times 10^{-2}$ mM, $c = 10^{-2}$, $\epsilon = 0.1$, $\epsilon' = \theta = 0$.

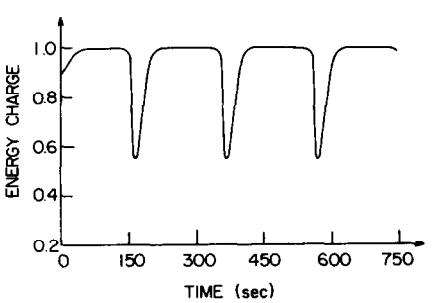

Fig. 3. Periodic variation of the energy charge. The curve is generated according to eq. (5) by numerical integration of equations (2a, b) on a digital computer, for $\sigma_1 = 5\, k_S$. Other parameters are as in fig. 2. At the unstable steady state ($\alpha = 40.198$, $\gamma = 5$), the value of the energy charge is 0.945 (see fig. 2).

The time variation of the adenylate charge for an injection rate located in the middle of the unstable domain is indicated in fig. 3. Sustained oscillations of the charge are observed, resulting from the limit cycle behavior in the α–γ phase plane. The charge is maintained close to unity on a plateau which extends over most of the period, and sharply drops then to 0.55. The plateau level exceeds the corresponding steady-state value of the charge. In the case considered, the period is around 200 sec and the duration of the fall approximately 30 sec. For longer periods, the latter phenomenon could last more than a minute. It should be stressed that the periodic variation of the energy charge depends on the substrate injection rate and on the characteristics of the enzyme. Indeed, the relative duration of the fall with respect to the period increases with σ_1, and its amplitude diminishes in a perfect K system (θ=1). Even in the latter case, the AEC oscillatory range is 0.75–1, which corresponds to the domain of steepest enzyme response to energy charge.

4. Discussion

The comparison of the energy charge at the unstable steady state and on the limit cycle suggests a possible function for metabolic oscillations involving the adenylate pool. At the steady state, the charge is close to 0.9 and favors mostly ATP-utilizing sequences. This phenomenon is enhanced on the plateau during oscillatory behavior, but the periodic drop in energy charge would allow temporary activation of energy-yielding processes and inhibition of those requiring ATP. Thus oscillations could give rise to an alternating operation of metabolic pathways. From a functional point of view, it is conceivable that periodic operation around an unstable stationary state allows greater flexibility with respect to cellular needs than steady-state processes in view of the broader metabolic range covered over a period.

Besides the kinetic control exerted by the energy charge, a similar effect can be brought about by the periodic variation in the affinity [13] of the hydrolysis reaction of ATP:

$$A = RT \ln \{K_{eq}\, [ATP]/[ADP]\, [P_i]\} \qquad (6)$$

which provides the driving force of most biosynthetic and active transport processes [16]. A simple calculation based on the variation of the ratio $(\alpha/\gamma) = [ATP]/[ADP]$ at constant phosphate concentration indicates that the difference between the affinity for ATP hydrolysis on the limit cycle and at the steady state oscillates in the range −2 to +2 kcal/mole, and that the mean value of this difference is generally positive. A detailed calculation can only be carried out in a complete model for glycolysis since phosphate, like all metabolites involved in this system, participates in the oscillations.

A consequence of what precedes is that the source term σ_1 in equation (2a), linked to the production of ATP, could be periodic rather than constant. In such a case, however, the system retains the main attributes of limit cycle behavior [15,17].

As previously noted, expression (5) does not include AMP and is not modified upon introduction of an equilibrium adenylate kinase reaction. This approximation is not unreasonable since AMP concentration often remains much smaller than those of other adenylates [2]. Furthermore, the theoretical amplitude of energy charge variation is in agreement with the range 0.63–0.9 observed during glycolysis in oscillating heart extracts [5]. In yeast, taking into account the 180° phase difference between ATP on one hand, and ADP, AMP on the other, the data for maximal and minimal concentrations of adenine nucleotides during oscillations [2] yield the range 0.7–0.93 in the extract and 0.8–0.95 in the cells. These figures can be matched in the model for larger values of the ratio of turnover numbers θ. The oscillatory range is shifted to lower values of the adenylate charge when AMP concentration is of the same order as that of ADP, as found in yeast extracts supplemented with a low input of substrate [4] in which the energy charge oscillates between 0.6 and 0.75.

It should be stressed that the total concentration of adenine nucleotides does not remain constant in the system, and varies by a factor 5 on a period in fig. 3. This results from the fact that the system has to be open for the limit cycle to occur. Enzyme responses to energy charge may be affected by the total adenylate concentration [18], and by a number of factors such as pH, non-adenine nucleotides [18] and non-nucleotide effectors [10].

As periodic behavior has also been observed in mitochondria [1,19] and photosynthesis [20], the results reported for glycolysis could bear on the other systems responsible for maintenance of the adenylate energy charge in vivo.

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