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MULTIPLICITY OF STEADY STATES AND AUTO-OSCILLATIONS IN THE OPEN REACTION CATALYSED BY PHOSPHOFRUCTOKINASE OF *E. COLI*. QUANTITATIVE MODEL*

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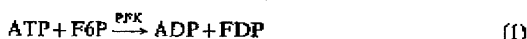
The authors have studied a quantitative mathematical model of an open two-substrate reaction catalysed by phosphofructokinase of *E. coli*. The fit of the parameters of the model of the phosphofructokinase reaction to experimental curves allows them to describe these data with an accuracy not worse than 5 per cent. The regions of the permissible values of the rates of exchange of fructose-6-phosphate, ATP and ADP at which alternative steady states and auto-oscillations exist in the reaction are defined. A minimal three-enzyme flow system in which these dynamic regimes may be observed experimentally is proposed.

PERIODIC CHANGES in the concentration of the intermediates of glycolysis observed in intact cells and cell-free extracts are being successfully explained by the regulatory properties of phosphofructokinase (PFK) (E.C. 2.7.1.11) [1-3]. However, so far there is no direct experimental confirmation of the periodic generation of ADP and fructose

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diphosphate (FDP) in this reaction. The appearance in experimental practice of membranes permeable for substrates with m.w. ~200 and practically impermeable for those with m.w. of 400 and more [4] makes it possible to organize a flow system with minimal set of enzymes for the experimental observation of periodic changes in the concentrations of substrates in the PFK reaction. The paper makes a preliminary quantitative calculation of the values of the rates of exchange of the substrates and products for which auto-oscillations in the system are possible and gives an evaluation of the period of these fluctuations.

Quantitative model of phosphofructokinase reaction. The experimental kinetic characteristics of the reaction



catalysed by PFK of *E. coli* are taken from [5] and presented in Fig. 1a. The mathematical model of the reaction (1) is constructed on the basis of the generalized model of Monod, Wyman and Changeux [6, 7] for the tetramer enzyme with a mechanism of rapid random attachment of F6P, ATP and ADP to the catalytic centres and binding of ADP at the allosteric centres:

$$v = V_{\max} \frac{[\text{F6P}][\text{ATP}]K_1 K_2}{d} \frac{1 + \alpha Lq^3}{1 + Lq^4} \quad (2)$$

where

$$q = d'/d, \quad L = L_0 [(1 + d[\text{ADP}]/K_3)/(1 + [\text{ADP}]/K_3^4)]^4$$

$$d = \left(1 + \frac{[\text{F6P}]}{K_1}\right) \left(1 + \frac{[\text{ATP}]}{K_2}\right) \left(1 + \frac{[\text{ADP}]}{K_3}\right)$$

$$d' = \left(1 + \frac{[\text{F6P}]}{K_1'}\right) \left(1 + \frac{[\text{ATP}]}{K_2'}\right) \left(1 + \frac{[\text{ADP}]}{K_3'}\right) \quad (3)$$

The fit of the parameters of the model (2) to the experimental data [5] using an algorithm [8] gave the following values of the parameters:

$$K_1 = 0.0092 \text{ mM}, \quad K_2 = 0.06 \text{ mM}, \quad K_3 = 0.18 \text{ mM}, \quad K_3' = 0.015 \text{ mM}, \quad (4)$$

$$K_1'/K_1 = c_1 = 0.003, \quad c_2 = 1, \quad c_3 = 0.25, \quad d = 0.011, \quad \alpha = 10^{-10}, \quad L_0 = 19 \times 10^6$$

The solid lines in Fig. 1a are plotted from the model (2) for values of the parameters of (4). The model quite well describes the experimental data, the greatest deviation of the theoretical value of the rate from the experimental does not exceed 5 per cent

Dynamics of the phosphofructokinase reaction in flow conditions. Let us consider the set of reactions



with the condition $[\text{ATP}] + [\text{ADP}] = A_0 = \text{const.}$ In this case the dependence of the rate:

of the PFK reaction v on ADP in the system (5) is described by the kinetic curves in Fig. 1b.

We consider that the rate of uptake of FDP is less than the rate of its splitting $v_2 - v$ so that $[FDP] \approx 0$ and its influence on the rate of the reaction of PFK may be

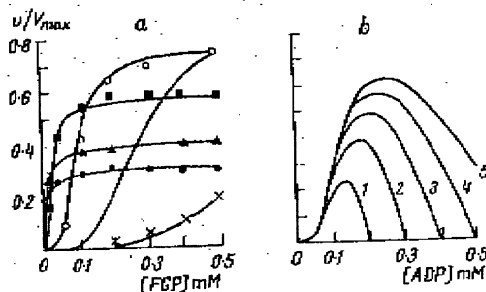


FIG. 1. *a* - Dependence of the initial rate of reaction catalysed by PFK of *E. coli* on concentration of the substrate F6P and product ADP for $ATP = 10^{-4} M$ and $Mg^{2+} = 10^{-3} M$ [5]. Concentration of ADP: \times - 0, \square - 0.02, \circ - 0.07, \blacksquare - 0.22, \blacktriangle - 0.52, \bullet - 0.82 mM. Solid lines are plotted from model (2) for values of the parameters (4). *b* - Dependence of rate of PFK reaction on concentration of ADP for $[ADP] + [ATP] = A_0$. Curves plotted from model (2) for $[F6P] = 0.05 M$ and different values of A_0 : 1) 0.2, 2) 0.3, 3) 0.4, 4) 0.5, 5) 0.6 mM.

ignored. The rate of uptake of F6P (v_1) and the regeneration of ATP are described by the equations

$$v_1 = v_{1m} - k_1 [F6P], \quad v_2 = V_2 [ADP] / (K_n + [ADP]) \quad (6)$$

Change in the concentrations $S \equiv [F6P]$, $A_1 \equiv [ATP]$, $A_2 \equiv [ATP]$ in the reactions (5) is described by the following set of equations:

$$\frac{dS}{dt} = v_1 - v \equiv F_1, \quad \frac{dA_2}{dt} = v - v_2 \equiv F_2, \quad A_3 = A_0 - A_2. \quad (7)$$

On fulfilment of the conditions

$$F_1 = F_2 = 0, \quad \Delta = \begin{vmatrix} \frac{\partial F_1}{\partial S} & \frac{\partial F_2}{\partial S} \\ \frac{\partial F_1}{\partial A_2} & \frac{\partial F_2}{\partial A_2} \end{vmatrix} > 0, \quad Sp = \frac{\partial F_1}{\partial S} + \frac{\partial F_2}{\partial A_2} > 0, \quad (8)$$

the set of equations (7) describes the sustained fluctuations of the concentrations of F6P, FDP, ATP and ADP. On fulfilment of the conditions

$$F_1 = F_2 = 0, \quad Sp > 0, \quad \Delta < 0 \quad (9)$$

in the system (7) the trigger regime is possible.

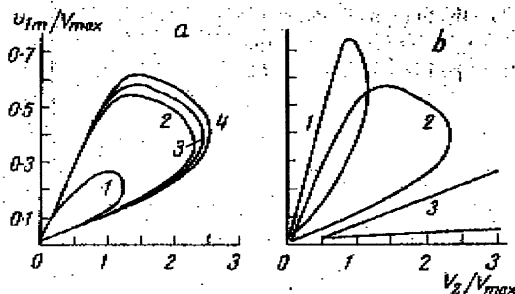


FIG. 2. Regions of values of relative rates of uptake of F6P (v_{1m}/V_{max}) and regeneration of ATP (V_2/V_{max}) for which in system (5) auto-fluctuations are possible for $k_1=0$. *a*— $K_m=0.05$ mM, $A_0=0.1$, 0.5, 1 and 2 mM (curves 1, 2, 3, 4). *b*— $A_0=1$ mM, $K_m=0.01$, 0.05, 0.5 mM (curves 1, 2, 3).

If the source of F6P is irreversible ($k_1=0$ in (6)) then always $\Delta > 0$ and in the system of reactions (5) only one steady state of the node or focus type (stable or unstable) is possible. The regions of the permissible values of the parameters of the flow system for which the steady state is unstable are given in Fig. 2. Numerical integration of the

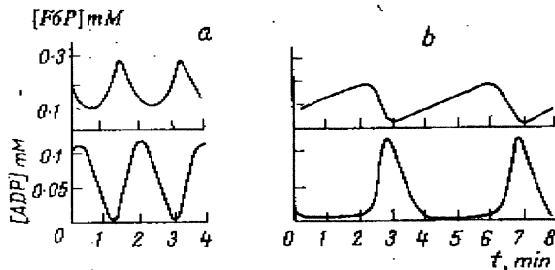


FIG. 3. Fluctuations in concentration of F6P and ADP obtained by integrating set of eqns. (7) for $k_1=0$, $K_m=0.05$ mM, $A_0=1$ mM, $V_{max}=1$ mM/min and: *a*— $v_{1m}/V_{max}=0.5$, $V_2/V_{max}=1$; *b*— $v_{1m}/V_{max}=0.07$, $V_2/V_{max}=0.3$.

set of equations (7) for $V_{max}=1$ mM/min and K_m , A_0 , V_2/V_{max} and v_{1m}/V_{max} taken from the regions of instability (Fig. 2*a*, *b*) gives the auto-oscillations of the concentrations of F6P, ATP, ADP and FDP with the period $T \sim 1-4$ min (Fig. 3).

If the source of F6P is reversible ($k_1 > 0$ in (6)) then on fulfilment of the conditions (8) in the system (5) auto-fluctuations are possible (region of the values of the parameters I in Fig. 4), on fulfilment of the conditions (9) there may be three steady states one of which is a saddle (region II) in Fig. 4). In this case trigger switching of the system from one steady state to another with slight change in the concentrations of the reagents is observed.

Discussion. The fluctuations in the concentrations of F6P, ATP and ADP obtained on quantitative analysis of the set of reactions (5) in form, amplitude and period (Fig. 3), correspond to the experimentally observed fluctuation in the complete glycolytic system [1, 2]. It is impossible to obtain an auto-oscillations regime on phosphofructokinase

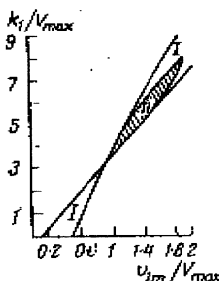


FIG. 4. Region of values of k_1/V_{max} and v_{1m}/V_{max} for which in reactions (5) auto-fluctuations are possible (region I) and trigger regime (region II). Boundaries of corresponding regions are constructed for $K_m=0.05$ mM, $A_0=1$ mM, $V_2/V_{max}=1$.

itself since the run-off of ADP without simultaneous escape of F6P is technically not feasible. The presence in modern experimental practice of lipid membranes with incorporated antibiotic amphotericin permeable for the substances of m.w.~200 and impermeable for those with one of 400 and more [4] makes it possible to organize the selective run-off of the products of the aldolase (E. C. 4.1.2.7) reaction (m.w. < 200) without escape of F6P, FDP, ATP and ADP (m.w. ~ 400). In this case the minimal system corresponds to the scheme (5) and includes three enzymes: PFK of activity V_{max} , aldolase with an activity $V_2 > V_{max}$ to avoid accumulation of FDP in the reaction volume and the enzyme regenerating ATP, for example, pyruvate kinase (E.C. 2.7.1.40) of activity V_2 chosen in line with Fig. 2. The membrane is permeable for the pyruvate formed. Through this system with a rate constant μ is passed the buffer solution [5] containing 10 mM $MgCl_2$, phosphoenol pyruvate and F6P in a concentration of v_{1m}/μ where v_{1m} is determined from Fig. 2.

For active aldolase the parameters of the fluctuations for the products of splitting of FDP correspond to those of F6P and the fluctuations of pyruvate to those of ADP. The maximum amplitude of the changes in F6P is determined by the kinetic properties of PFK and does not exceed $\sqrt{4/L_0 K_1} \approx 0.4$ mM, $[ADP] \leq A_0$. The period of fluctuations for $V_2 = V_{max}$ ($=1$ mM/min) and $v_{1m} \approx V_{max}/2$:

$$T \geq \frac{4 \{ [F6P]_{max} - [F6P]_{min} \}}{V_{max}} \approx 0.8 \text{ min.}$$

To obtain the trigger regime, to the existing system of enzymes one may add an enzyme of activity k_1 carrying out the irreversible conversion of F6P to a product not influencing PFK, PK and aldolase. Such an enzyme may be for example hexo-

phosphate of aminotransferase (E.C.2.6.1.16). By regulating the rate of the flow larger values of μ^* and from smaller values to μ^* it is possible to observe at rate μ^* determined from Fig. 4 the establishment in the system of reactions of stable steady states with high or low concentrations of products.

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EFFECT OF CONCAVALINE A ON THE ADSORPTION OF CALCIUM IONS BY LYMPHOCYTES*

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Concavaline A induces incorporation of calcium into the lymphocytes of peripheral blood and increases the intensity of the fluorescence of chlortetracyclin forming complex with calcium on the outer surface of the plasma membranes.

THE INTERACTION of mitogens with the molecules of specific receptors on the surface of lymphocytes is accompanied by rearrangements of the components of plasma membranes and metabolic changes [1]. In particular, an important role in the activation of the lymphocytes is ascribed to their mitogen-induced uptake of calcium ions usually measured by the radio-isotope method [2]. The increase thus observed in ^{45}Ca associated with the cells may be the result of its transport into the and/or adsorption of calcium ions on the surface of the plasma membrane

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