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

We consider that the rate of uptake of FDP is less than the rate of its splitting $v_3 - v$ so that [FDP]≈0 and its influence on the rate of the reaction of PFK may be

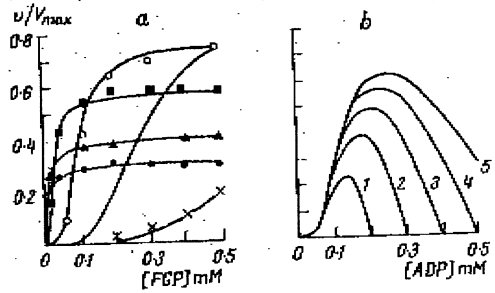

FIG. 1. *a* – Dependence of the initial rate of reaction catalysed by PFK of *E. coli* on concentration of the substrate F6P and product ADP for ATP = 10^{-4} M and $Mg^{2+} = 10^{-2}$ M [5]. Concentration of ADP: × – 0, □ – 0·02, ○ – 0·07, ■ – 0·22, ▲ – 0·52, ● – 0·82 mM. Solid lines are plotted from 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

$$v_1 = v_{1m} - k_1[\mathrm{F6P}], \qquad v_2 = V_2[\mathrm{ADP}]/(K_m + [\mathrm{ADP}]) \tag{6}$$

Change in the concentrations $S \equiv [\mathrm{F6P}]$, $A_2 \equiv [\mathrm{ATP}]$, $A_3 \equiv [\mathrm{ATP}]$ in the reactions (5) is described by the following set of equations:

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

On fulfilment of the conditions

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

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

$$F_1 = F_2 = 0, \qquad Sp > 0, \qquad \Delta < 0 \tag{9}$$

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

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

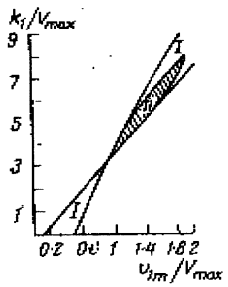

Fig. 4. Region of values of k_1/V_{max} and v_{1m}/V_{max} for which in reactions (5) auto-fluctuations are possible (region I) and trigger regime (region II). Boundaries of corresponding regions are constructed for $K_m = 0{\cdot}05$ mM, $A_0 = 1$ mM, $V_2/V_{max} = 1$.

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 $V_3 > V_{max}$ 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 u is passed the buffer solution [5] containing 10 mM $MgCl_2$, phosphoenol pyruvate and F6P in a concentration of v_{1m}/u 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 $\sim\sqrt[4]{L_0 K_1} \approx 0{\cdot}4$ mM, $[ADP] \leqslant A_0$. The period of fluctuations for $V_2 = V_{max}$ (=1 mM/min) and $v_{1m} \approx V_{max}/2$:

$$T \geqslant \frac{4\{[F6P]_{max} - [F6P]_{min}\}}{V_{max}} \approx 0{\cdot}8 \text{ min}.$$

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-