ON THE POSSIBILITY OF SELF-OSCILLATIONS IN THE LOWER PART OF THE GLYCOLYTIC SYSTEM

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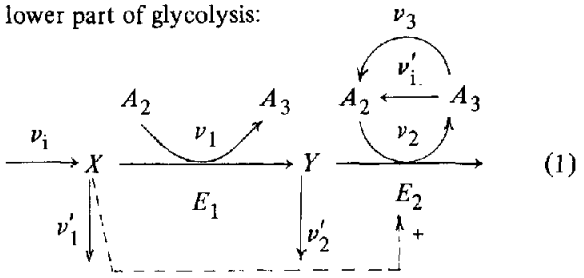
1. Introduction

The single-frequency periodicity that occurs at the phosphofructokinase step of the glycolytic system is well studied both experimentally [1–6] and theoretically [7–10]. Along with single-frequency oscillations, a double-frequency periodicity was observed in some experiments [2–5], this suggesting the existence of another self-generator which is only slightly connected with the phosphofructokinase reaction. This paper presents an attempt to analyse theoretically the conditions for the existence of a second self-generator in the glycolytic system.

2. Kinetic model

Consider a very simplified kinetic model of the lower part of glycolysis:



(1)

Here X stands for the overall substrate pool consisting of fructose 1,6-diphosphate (FDP), glyceraldehydephosphate and dihydroxyacetonephosphate; Y stands for the pool consisting of 3-phosphoglycerate, 2-phosphoglycerate and phosphoenolpyruvate; A_2 and A_3 stand for ADP and ATP; E_1 is the enzyme whose effect is equivalent to the total catalytic activity of aldolase (EC 4.1.2.13), triose phosphate isomerase (EC 5.3.1.1), glyceraldehydephosphate dehydrogenase (GAPDH, EC 1.2.1.12), and phosphoglycerate kinase (PGK, EC 2.7.2.3); E_2 the enzyme whose effect is equivalent to the catalytic activity of phosphoglycerate mutase (EC 2.7.5.3), enolase (EC 4.2.1.11), and pyruvate kinase (EC 2.7.1.40); ν_i the rate of production of X; ν_i' the rate of the A_3 to A_2 conversion coupled with the production of $X(\nu_i = n\nu_i'$, where $n > 1$ is the stoichiometric coefficient); ν_1 and ν_2 the rates of the reactions catalysed by E_1 and E_2; ν_1' and ν_2' the rates of leakage of X and Y into non-glycolytic pathways; ν_3 the rate of A_3 utilization through the 'ATPase' reaction.

Let the following assumptions hold for model (1):
1) The rates of separate reactions can be approximated by the expressions:

$$\nu_i = n\nu_i' = \text{const}, \tag{2}$$
$$\nu_1 = k_1[X][A_2], \tag{3}$$
$$\nu_1' = k_1'[X], \tag{4}$$
$$\nu_2 = k_2[Y][A_2](\alpha + ([X]/K_a)^\gamma), \tag{5}$$
$$\nu_2' = k_2'[Y], \tag{6}$$
$$\nu_3 = k_3[A_3], \tag{7}$$

where k_i are the rate constants, K_a and γ the activation constant and the order of the E_2 activation by X, resp.; $\alpha \ll 1$ the dimensionless parameter which determines the relative activity of E_2 at $[X] = 0$.
2) The pool of the coenyzmes is constant

$$A_2 + A_3 = A_0 = \text{constant} \tag{8}$$

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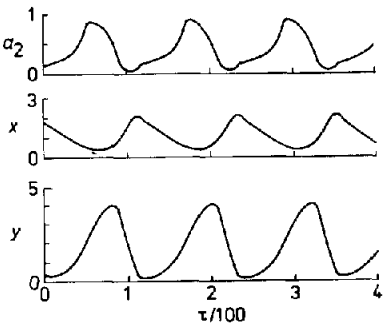

Fig. 1. Oscillations, with time τ, in the concentrations of $X(x)$, $Y(y)$ and $A_2(a_2)$ obtained by integration of model (9) at $\epsilon_1 = 3$, $\epsilon_2 = 3$, $\gamma = 4$, $\nu_i = 0.16$, $\nu_i' = 0.08$, $\nu_0 = 0.01$, $\beta_1 = 0.2$, $\beta_1 = 0$, $\beta_3 = 0.2$. For integration the Runge–Kutta method in Merson's modification was used. The relative error of integration was less than 0.1%.

3) The reactions of model (1) run under conditions of ideal mixing with pH and temperature kept constant.

With due regard for the above assumptions, reaction system (1) may be described by the following mathematical model of the third order:

$$\left.\begin{aligned} \epsilon_1 \frac{dx}{d\tau} &= \nu_i - \beta_1 x - a_2 x, \\ \frac{dy}{d\tau} &= a_2 x - a_2 y(\nu_0 + x^\gamma) - \beta_2 y, \\ \epsilon_2 \frac{da_2}{d\tau} &= \nu_i' + \beta_3 a_3 - a_2 x - a_2 y(\nu_0 + x^\gamma). \end{aligned}\right\} \quad (9)$$

All the variables and parameters in this model are dimensionless:

$$x = \frac{[X]}{k_a}, \; y = \frac{k_2[Y]}{k_1 K_a}, \; a_2 = \frac{[A_2]}{A_0}, \; \nu_0 = \alpha,$$

$$\tau = tk_2A_0, \; \nu_i = \frac{\nu_i}{k_1 K_a A_0}, \; \nu_i' = \frac{\nu_i'}{k_1 K_a A_0}, \; \beta_1 = \frac{k_1'}{k_1 A_0}, \quad (10)$$

$$\beta_2 = \frac{k_2'}{k_2 A_0}, \; \beta_3 = \frac{k_3}{k_1 K_a}, \; \epsilon_1 = \frac{k_3}{k_1}, \; \epsilon_2 = \frac{A_0}{K_a}\frac{k_2}{k_1}$$

As follows from analysis of model (9), it can reveal under definite conditions the multiplicity of alternative stationary states and self-oscillations. One of possible oscillatory solutions of the model obtained with a computer is shown in fig. 1.

3. Oscillatory mechanism

Analysis of model (9) becomes much easier if a_2 is a fast variable. This is possible when $a_2 \ll 1$ or $\epsilon_2 \ll 1$. In the second case the condition

$$\frac{k_2}{k_1} \ll \frac{K_a}{A_0} \quad (11)$$

should be fulfilled which demands that the activity of E_2 be lower in comparison with E_1. Making use of assumption (11), we shall pass to the limit $\epsilon_2 \to 0$ with the result that model (9) will reduce to the second order model

$$\left.\begin{aligned} \epsilon_1 \frac{dx}{d\tau} &= \nu_i - \beta_1 x - \tilde{\nu}_1, \\ \frac{dy}{d\tau} &= \tilde{\nu}_1 - \tilde{\nu}_2 - \beta_2 y \end{aligned}\right\} \quad (12)$$

in which

$$\tilde{\nu}_1 = \frac{(\nu_i' + \beta_3)x}{\beta_3 + x + y(\nu_0 + x^\gamma)} \quad (13)$$

and

$$\tilde{\nu}_2 = \frac{y(\nu_i' + \beta_3)(\nu_0 + x^\gamma)}{\beta_3 + x + y(\nu_0 + x^\gamma)} \quad (14)$$

are the dimensionless quasi-stationary expressions for the rates ν_1 and ν_2.

As follows from equation (13), at y = const and $\gamma > 1$ the rate $\tilde{\nu}_1$ has a maximum

$$x = x_{max} \equiv \sqrt[\gamma]{\frac{\beta_3 + \nu_0 y}{(\gamma - 1)y}} \quad (15)$$

because of this, at $x > x_{max}$ the rate $\tilde{\nu}_1(x)$ falls with increasing x and y = const. Paradoxical as it may seem from the first glance, this effect can be explained by competition between E_1 and E_2 for their common

cofactor A_2. At y = const, the cooperative ($\gamma > 1$) activation of E_2 by X results in that the enzyme E_2 turns out to be more active at sufficiently high levels of X thus winning in a competition for A_2. As a result, the rate $\tilde{\nu}_1$ falls as the rate $\tilde{\nu}_2$ increases. This explanation is also valid in the case where the value of y is not exactly constant but changes slowly (as compared with the variable x) with time. Such a discrepancy in the rates of change of the variables x and y occurs when

$$\epsilon_1 \ll 1 \tag{16}$$

or

$$k_2 \ll k_1. \tag{17}$$

In this case, as is readily apparent from model (12),

$$\frac{dy}{dx} \sim \epsilon_1 \ll 1,$$

i.e. y changes ϵ_1 times as slow as x.

Thus, at $\epsilon_1, \epsilon_2 \ll 1$ and $\gamma > 1$ the reaction catalysed by the enzyme E_1 is subject to an apparent (kinetic) inhibition by excess of the substrate X. An immediate result of this inhibition is the hysteretic dependence [11] of the rate $\tilde{\nu}_1$ on y in a quasi-stationary state in which

$$\frac{dx}{d\tau} = 0. \tag{18}$$

or

$$\nu_i - \beta_1 x - \tilde{\nu}_1(x,y) = 0. \tag{19}$$

So long as this state is stable, the value of the variable x is very close to the root of equation (19), $x = \tilde{x}$. Denoting the quasi-stationary value of $\tilde{\nu}_1$ by $\tilde{\tilde{\nu}}_1$,

$$\tilde{\tilde{\nu}}_1 = \tilde{\nu}_1(\tilde{x},y) \tag{20}$$

and making use of the condition

$$\tilde{\tilde{\nu}}_1 = \nu_i - \beta_1 \tilde{x}, \tag{21}$$

we obtain from equation (19) an explicit dependence of y on $\tilde{\tilde{\nu}}_1$:

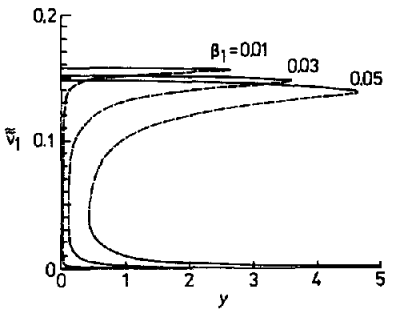

Fig. 2. The family of quasi-stationary output characteristics of the reaction catalysed by the enzyme E_1. y Is the dimensionless concentration of Y, $\tilde{\tilde{\nu}}_1$ the relative quasi-stationary rate of the reaction. Broken line shows the unstable values of the quasi-stationary rate. Curves were derived from equation (22) for $\gamma = 4$, $\nu_0 = 0.02$, $\nu_i = 0.16$, $\nu_i' = 0.08$, $\beta_2 = 0$, $\beta_3 = 0.2$ and for a few values of the parameter β_1 as shown in the figure.

$$y = \frac{\beta_1^{\gamma-1}}{\tilde{\tilde{\nu}}_1}\left(\frac{(\tilde{\tilde{\nu}}_1 - \nu_i)(\nu_i' + \beta_3 - \tilde{\tilde{\nu}}_1) - \beta_1\beta_3\tilde{\tilde{\nu}}_1}{(\tilde{\tilde{\nu}}_1 - \tilde{\tilde{\nu}}_i)^\gamma + \beta_1\nu_0}\right). \tag{22}$$

Specifying the values of $\tilde{\tilde{\nu}}_1$ and calculating the values of $y(\tilde{\tilde{\nu}}_1)$ from equation (22), we can obtain a quasi-stationary output characteristics of the reaction catalysed by the enzyme E_1, i.e. the fubction $\tilde{\tilde{\nu}}_1 = \tilde{\tilde{\nu}}_1(y)$. The family of such output characteristics is shown in fig. 2. As seen from this figure, the output characteristics display a hysteretic character. It is due to

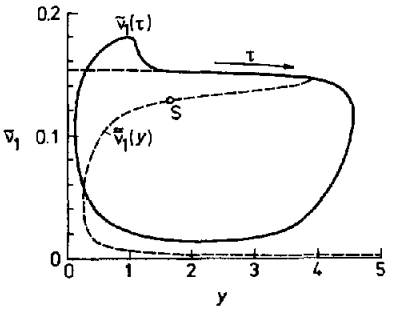

Fig. 3. Limit cycle in the $(\tilde{\nu}_1, y)$-plane of model (12) surrounding the unstable stationary point S which belongs to the unstable part of the output characteristics, $\tilde{\tilde{\nu}}_1(y)$. The limit cycle was obtained by numerical integration of model (12) by means of the Runge–Kutta–Merson method. The relative error of integration was less than 0.1%. Curves were derived for $\epsilon_1 = 10$, $\epsilon_2 = 10$, $\nu_0 = 0.01$, $\nu_i = 0.16$, $\nu_i' = 0.08$, $\beta_1 = 0.04$, $\beta_2 = 0$, $\beta_3 = 0.2$, $\gamma = 4$.

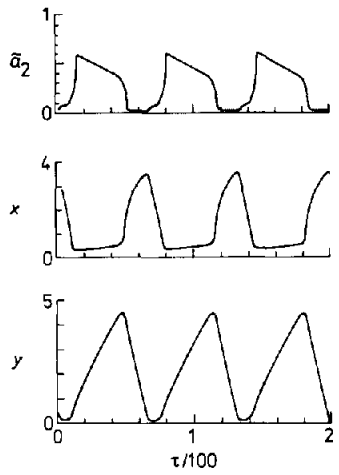

Fig. 4. Oscillations, with time τ, in the variables of model (12) due to the limit cycle shown in the above figure. Quasi-stationary a_2 as a function of time τ was calculated with the help of the quasi-stationary equation:

$$\tilde{a}_2 = \frac{\nu_1 + \beta_3}{\beta_3 + x + y(\nu_0 + x\gamma)}$$, which follows from system (9) as $\epsilon_2 \to 0$.

this hysteretic phenomenon that system (1) can yield oscillations when the stationary point ($dx/d\tau = dy/d\tau = 0$) is located on the unstable part of the output characteristics. Fig. 3 shows an output characteristics with a stable limit cycle surrounding the unstable stationary point S. This limit cycle was obtained by solving numerically model (12). The change with time in the variables of model (14) is shown in fig. 4.

4. Discussion

As follows from analysis of model (12), a rapid increase in the FDP concentration above some critical value will lead to a temporary inhibition of the PGK reaction. This inhibition which results in an increase of the diphosphoglycerate concentration must cause a drop in the rate of the GAPDH reaction and a rise in the level of NAD. If reoxidation of the glycolytic NADH occurs primarily at the expense of pyruvate or acetaldehyde reduction, the increase in the level of FDP, which is an activator of pyruvate kinase, must contribute to an accelerated formation of pyruvate and to intensification of the NADH reoxidation. This must of necessity cause an additional increase in the NAD level. Hess (personal communication) showed that the addition of FDP to the glycolytic system reconstituted from purified yeast enzymes actually led to a sharp temporary increase in the NAD level which seems to speak well for our model. However, as it is difficult to distinguish from this experiment between the contributions to the effect of PGK and GAPDH inhibition and of an accelerated reoxidation of NAD, it might be as well to repeat it in the conditions under which the NAD reoxidation would be independent on the pyruvate level. For instance, the NAD reoxidation will be insensitive over a certain period of time to a changing activity of pyruvate kinase if pyruvate (or acetaldehyde) is supplied in a large excess. If in *this* case the addition of FDP leads to an increase in the NAD level, this will point more clearly toward the inhibiting action of FDP on PGK and GAPDH.

We do not think that the allosteric cooperative activation of pyruvate kinase by FDP is the only regulation responsible for a hysteretic character of the function $\tilde{\nu}_1(y)$ and thus for self-oscillations in the lower part of the glycolytic system. Most likely, this hysteresis as well as that of the input characteristics of the phosphofructokinase reaction [9, 10] is due to a concerted action of many equivalent mechanisms. One of these, for instance, may be the inhibition of GAPDH by glyceraldehyde-3-phosphate [12–14] which can cause self-oscillations in the lower part of the glycolytic system even in the absence of pyruvate kinase activation by FDP. The possibility of participation of this mechanism in the generation of glycolytic oscillations was first discussed by Betz and Chance [15].

Acknowledgement

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