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Self-Oscillations in Glycolysis

1. A Simple Kinetic Model

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The paper describes a simple kinetic model of an open monosubstrate enzyme reaction with substrate inhibition and product activation. A comparison between the model and the phosphofructokinase reaction shows a close resemblance between their dynamical properties. This makes it possible to explain qualitatively most experimental data on single-frequency oscillations in glycolysis. A mathematical analysis of the model has shown the following.

1. In the model, at a definite relationship between the parameters, self-oscillations arise.

2. The condition of self-excitation is satisfied more readily with a lower source rate, larger product sink rate constants, lower product-enzyme affinity and higher enzyme activity.

3. Self-oscillations exist only in a certain range of values of the parameter determining the degree of substrate inhibition. This range increases with decreasing source rate. Too strong or, conversely, too weak substrate inhibition leads to damped oscillations.

4. The period of self-oscillations depends on the degree of substrate inhibition, the source rate, the sink rate constant, the enzyme activity, the affinity of the substrate and the product for the enzyme; it decreases with an increase in these values.

5. With an increase in the relative sink rate constant the steady state amplitude of self-oscillations initially increases until a definite maximum is reached and then drops to zero.

6. A self-oscillatory state in the phosphofructokinase reaction exists only when the maximum rate of this reaction is essentially higher than the source rate, and lower than the maximum rate of the reactions controlling the sink of the products.

7. An experimental investigation of self-oscillations in the phosphofructokinase reaction may be considerably simplified by using a reconstituted system consisting of a small number of reactions with an irreversible sink of the products and artificial substrate supply. In this case the above relationship (section 6) should hold.

Periodical oscillations in biochemical systems are receiving much attention nowadays. A review of studies dealing with this problem can be found in [1]. The interest in this phenomenon has developed rapidly since the existence of sustained oscillations in yeast cell extracts [1—9] and in the whole yeast cell [10] as well as in heart muscle cell extracts [11] has been proved experimentally.

The phosphofructokinase reaction is commonly thought of as being a possible source of self-oscillations in glycolysis. However, the conditions for the appearance of self-oscillations have not been ascertained. There is a considerable number of scattered experimental facts which have not been united by a general scheme.

Higgins [12,13] has presented a model to explain sustained oscillations in the yeast glycolytic system. His model, however, as will be shown later, has no limit cycle for those values of its parameters with which self-oscillations are observed experimentally.

This paper describes a simple kinetic model which qualitatively explains most of the experimental facts concerning single-frequency oscillations in glycolysis. The model represents an enzyme reaction with substrate inhibition and product activation. These properties are shown to be common for phosphofructokinase from different sources [14—23].

Enzymes. Adenylate kinase or ATP: AMP-phosphotransferase (EC 2.7.4.3); apyrase or ATP diphosphohydrolase (EC 3.6.1.5); α-glucanphosphorylase or α-1,4-glucan: orthophosphate—glucosyltransferase (EC 2.4.1.1), hexokinase or ATP: D-hexose 6-phosphotransferase (EC 2.7.1.1), phosphoglycerate kinase or ATP: 3-phospho-D-glycerate-1-phosphotransferase (EC 2.7.2.3), phosphofructokinase or ATP: D-fructose-6-phosphate 1-phosphotransferase (EC 2.7.1.11), pyruvate kinase or ATP: pyruvate phosphotransferase (EC 2.7.1.40).

THEORY

Preliminary Considerations

Starting from the fact that phosphofructokinase is an important control site in the glycolytic system

let us try to determine those properties of the phosphofructokinase reaction which could account for the appearance of self-oscillations in this system.

In spite of some differences, all the phosphofructokinases obtained from various sources are known to be strongly inhibited by one of the substrates, ATP [14—23]. Although direct evidence for the existence of such an inhibition in cell extracts or in the whole cells has not been obtained it can be assumed that it takes place there as well.

Different phosphofructokinases also all show an activation by the products, ADP [15,17,20,21,23] and fructose-1,6-diphosphate [14—22]. It is to be noted, however, that the level of fructose-1,6-diphosphate in yeast cell extracts showing self-oscillations [4—9] is very high, approaching 10 mM [9]. At such a concentration it is not activating [17,19,21] and therefore need not be considered.

It has been shown [9] that in oscillating cell-free extracts of yeast *Saccharomyces carlsbergensis* the concentration of fructose-6-phosphate undergoes only slight changes, making it possible to assume that the concentration remains constant.

And finally, in those cases where extracts or whole cells show a considerable adenylate kinase activity a very important factor determining properties of phosphofructokinase is the concentration of AMP. Different phosphofructokinases are known to be strongly activated by AMP [15—22] (with the exception of that from *Escherichia coli* [23]). At high adenylate kinase activity the concentration of AMP must follow the changes in the concentration of ADP, and its activating effect on phosphofructokinase must be indistinguishable from that of ADP. Therefore, for the sake of simplicity, the total activating effect of ADP and AMP on phosphofructokinase may be attributed to the effect of ADP alone, and AMP need not be considered.

The above considerations lead us to a very simple kinetic model of the phosphofructokinase reaction. This model should represent a monosubstrate and monoproduct reaction, the enzyme of which is inhibited by the substrate and activated by the product.

Simple Kinetic Model

Consider a simple kinetic model of enzyme catalysis with product activation of the enzyme:

$$\left.\begin{array}{l} \xrightarrow{v_1} S_1 + ES_2^\gamma \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} S_1ES_2^\gamma \\ S_1ES_2^\gamma \xrightarrow{k_{+2}} ES_2^\gamma + S_2 \xrightarrow{v_2} \\ \gamma S_2 + E \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} ES_2^\gamma \,. \end{array}\right\} \quad (I)$$

Here the substrate S_1 (ATP) supplied by a certain source at the rate v_1 = constant is irreversibly converted to the product S_2 (ADP). The product is removed by an irreversible sink at the rate v_2. The free enzyme E (phosphofructokinase) is inactive by itself but becomes active combining with γ product molecules to form the complex ES_2^γ. Assume that reaction (I) proceeds in an ideally mixed medium.

Let us introduce certain notations for future convenience: $s_1 \equiv [S_1]$, $s_2 \equiv [S_2]$, $e \equiv [E]$, $x_1 \equiv [ES_2^\gamma]$, $x_2 \equiv [S_1ES_2^\gamma]$.

Let in (I) the following three conditions be satisfied:

$$\gamma > 1\,; \quad (1)$$

$$\frac{k_{+1}}{s_1},\ k_{-1},\ k_{+2},\ \frac{k_{+3}}{s_2^\gamma},\ k_{-3} \gg 1\,; \quad (2)$$

$$\frac{s_1}{e_0},\ \frac{s_2}{e_0} \gg 1\,, \quad (3)$$

where $e_0 \equiv e + x_1 + x_2$. The meaning of condition (1) will be made clear later. Condition (2) is common for most enzyme reactions. Condition (3) arises from the fact that the concentrations of the substrates and products in the phosphofructokinase reaction are of the order of 1 mM [9,24—26] whereas the enzyme concentration is far smaller, namely of the order of 10 μM [27,28], 1 μM [29] and even less [29,30].

Assume, also, that the sink of the product is a first order reaction:

$$v_2 = k_2 s_2.$$

This assumption is based on the fact that the rate of the glycolytic flux in a self-oscillatory state is considerably lower than the maximum rates of the reactions controlling the sink of the products of the phosphofructokinase reaction [6—9].

Mathematical Model

According to the law of mass action and the law of mass conservation reaction (I) is described by the equation system:

$$\left.\begin{array}{l} \dfrac{ds_1}{dt} = v_1 - k_{+1} s_1 x_1 + k_{-1} x_2 \\ \dfrac{ds_2}{dt} = k_{+2} x_2 - k_{+3} s_2^\gamma e + k_{-3} x_1 - k_2 s_2 \\ \dfrac{dx_1}{dt} = -k_{+1} s_1 x_1 + (k_{-1} + k_{+2}) x_2 + k_{+3} s_2^\gamma e - k_{-3} x_1 \\ \dfrac{dx_2}{dt} = k_{+1} s_1 x_1 - (k_{-1} + k_{+2}) x_2 \\ \dfrac{de}{dt} = -k_{+3} s_2^\gamma e + k_{-3} x_1 \end{array}\right\} \quad (4)$$

where t is time.

By substitution of the variables in accordance with conditions (2) and (3) [31,32] system (4) is reduced to the form in which the last three derivatives have a small factor ε. According to the Tikhonov's theorem [33,34] such a system can be replaced by

an asymptotical approximation resulting from the limit transition $\varepsilon \to 0$[1]:

$$\left.\begin{aligned} \frac{d\sigma_1}{d\theta} &= \nu_1 - \frac{\sigma_1\sigma_2^\gamma}{1+\sigma_2^\gamma(1+\sigma_1)} \\ \frac{d\sigma_2}{d\theta} &= \alpha_2\left[\frac{\sigma_1\sigma_2^\gamma}{1+\sigma_2^\gamma(1+\sigma_1)} - \varkappa_2\sigma_2\right] \end{aligned}\right\} \quad (5)$$

where

$$\left.\begin{aligned} &\sigma_1 \equiv \frac{k_{+1}s_1}{k_{-1}+k_{+2}},\ \nu_1 \equiv \frac{v_1}{k_{+2}e_0},\ \alpha_2 \equiv \frac{k_{-1}+k_{+2}}{k_{+1}}\left(\frac{k_{+3}}{k_{-3}}\right)^{\frac{1}{\gamma}}, \\ &\sigma_2 \equiv \left(\frac{k_{+3}}{k_{-3}}\right)^{\frac{1}{\gamma}}s_2,\ \varkappa_2 \equiv \frac{k_2}{k_{+2}e_0}\left(\frac{k_{-3}}{k_{+3}}\right)^{\frac{1}{\gamma}},\ \theta \equiv \frac{k_{+1}k_{+2}e_0}{k_{-1}+k_{+2}}t. \end{aligned}\right\} \quad (6)$$

Here σ_1 and σ_2 are relative concentrations of the substrate and product respectively, ν_1 is relative source rate, α_2 is relative enzyme-product affinity and θ is dimensionless time. The expression

$$\nu \equiv \frac{\sigma_1\sigma_2^\gamma}{1+\sigma_2^\gamma(1+\sigma_1)} \quad (7)$$

is a relative quasi-steady state rate of the reaction.

System (5) in a finite part of the phase plane has only one equilibrium state with the coordinates

$$\left.\begin{aligned} \bar{\sigma}_1 &= \frac{\varkappa_2^\gamma + \nu_1^\gamma}{\nu_1^{\gamma-1}(1-\nu_1)}, \\ \bar{\sigma}_2 &= \frac{\nu_1}{\varkappa_2}. \end{aligned}\right\} \quad (8)$$

Since in the equilibrium state $\nu = \nu_1$, equation (8) can be given as

$$\bar{\sigma}_1 = \frac{\varkappa_2^\gamma + \nu^\gamma}{\nu^{\gamma-1}(1-\nu)}. \quad (9)$$

As seen from equation (9), the function $\nu = \nu(\bar{\sigma}_1)$ has two asymptotes, $\nu = 0$ and $\nu = 1$. The plot of the function constructed by equation (9) for $\gamma > 1$ is shown in Fig. 1. The bottom branch of the plot reflects the presence of substrate inhibition.

At $\nu \ll 1$ and $\nu \ll \varkappa_2$ the function $\nu = \nu(\bar{\sigma}_1)$ can be approximated to the expression:

$$\nu = \frac{\varkappa_2^{\frac{\gamma}{\gamma-1}}}{\bar{\sigma}_1^{\frac{1}{\gamma-1}}} \quad (10)$$

from which it is seen that the degree of substrate inhibition increases as $\gamma \to 1$ on the right. As $\gamma \to \infty$ the inhibition is negligible.

[1] It is not difficult to see that the conditions of the theorem [33, 34] are satisfied in system (4).

Thus, for kinetic (I) and mathematical (5) models to take account of both product activation and substrate inhibition it is necessary that γ be greater than 1. However, the stoichiometry of product activation of the phosphofructokinase reaction is known to correspond to the first order ($\gamma = 1$). So for models (I) and (5) to take into consideration the most important properties of the phosphofructokinase reaction it is necessary to give up a pure kinetic

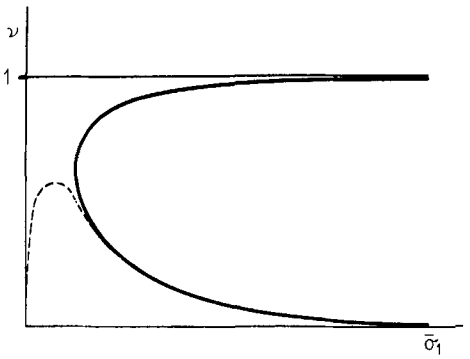

Fig. 1. *The relative reaction rate, ν, as a function of the relative steady state substrate concentration, $\bar{\sigma}_1$, at constant γ and $\varkappa_2$ and $\bar{\sigma}_2 = \nu/\varkappa_2$.* A dashed line shows the relationship between ν and $\bar{\sigma}_1$ in a substrate-inhibited reaction

interpretation of γ as a stoichiometric number of product activation and to consider it as a mathematical value determining both the degree of product activation and that of substrate inhibition. In this case the parameter γ can assume any value (including fractional) within the limits $1 < \gamma < \infty$.

The value of γ can be determined from experimental data in different ways depending on the parameter α_2. In particular, if $\alpha_2 \gg 1$ then γ is determined by the steady state input characteristics "rate-substrate concentration", obtained experimentally. At $\alpha_2 \ll 1$, γ is determined by the steady state output characteristics "rate-product concentration". And finally, at $\alpha_2 \sim 1$ the simplest way is to calculate γ by the equation given below which relates the period of oscillations to γ, the former being found from experiment.

Since self-oscillations in glycolysis are observed at a very low rate of the glycolytic flux [6—9] it is quite reasonable to consider system (5) at $\nu_1 \ll 1$. Under this condition expression (7) is simplified:

$$\nu \cong \sigma_1\sigma_2^\gamma.$$

Taking this into consideration, system (5) can be written as

$$\left.\begin{aligned} \frac{dx}{d\tau} &= 1 - x\,y^\gamma \\ \frac{dy}{d\tau} &= \alpha\,y\,(x\,y^{\gamma-1} - 1) \end{aligned}\right\} \quad (II)$$

where

$$\left.\begin{aligned} x &\equiv \nu_1^{\gamma-1}\varkappa_2^{-\gamma}\sigma_1, \quad y \equiv \nu_1^{-1}\varkappa_2\sigma_2, \\ \alpha &\equiv \alpha_2\nu_1^{-\gamma}\varkappa_2^{\gamma+1}, \quad \tau \equiv \nu_1^{\gamma}\varkappa_2^{-\gamma}\theta. \end{aligned}\right\} \quad (11)$$

At $\gamma > 1$ system (II) represents a mathematical model of a product-activated and substrate-inhibited reaction. Note that (II) is a generalization of the Lotka system [35] and coincides with it at $\gamma = 1$.

0_2 is a saddle-node with a parabolic sector in a positive quadrant; the ω-separatrix [37] of the saddle tends to 0_2 in the direction coinciding with the x-axis, and the α-separatrix [37] coincides with the equator of the Poincaré sphere [36,37]. The integral curves of the node sector enter 0_2 as $\tau \to \infty$. According to [37, p. 379] point 0_3 is a topological saddle at $\gamma = 2k+1$ and a saddle-node at $\gamma = 2k$. The neighbourhood of

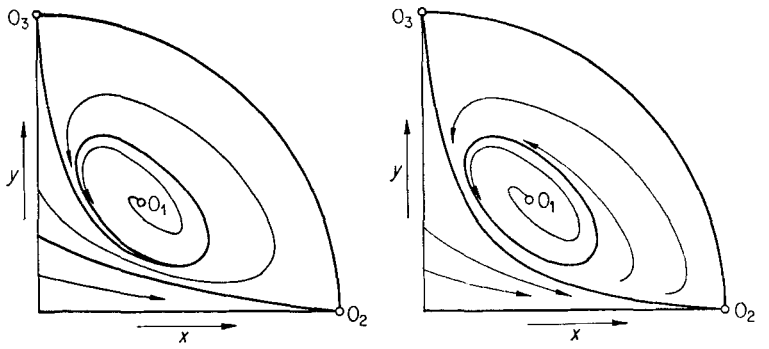

Fig. 2. *An orthogonal projection of a positive octant of the Poincaré sphere on a plane: possible types of the phase portraits of system (II) when 0_1 is an unstable focus.* x and y are dimensionless concentrations of the substrate and product, respectively

Conditions for Self-Oscillations in System (II)

In a finite part of the (x, y) phase plane system (II) has only one equilibrium state, 0_1, with the coordinates $\bar{x} = \bar{y} = 1$. In the neighbourhood of 0_1, system (II) has a characteristic equation:

$$\begin{vmatrix} -1-\lambda & -\gamma \\ \alpha & \alpha(\gamma-1)-\lambda \end{vmatrix} = 0,$$

its roots being

$$\lambda_{1,2} = \frac{1}{2}\{\alpha(\gamma-1) - 1 \pm \sqrt{[\alpha(\gamma-1)-1]^2 - 4\alpha}\}.$$

Hence it follows that 0_1 is

a stable node at	$0 < \alpha \leqslant \alpha_1$,
a stable focus at	$\alpha_1 < \alpha < \alpha_0$,
an unstable focus at	$\alpha_0 < \alpha < \alpha_2$,
an unstable node at	$\alpha_2 \leqslant \alpha < \infty$.

Here

$$\alpha_0 \equiv \frac{1}{\gamma-1}, \quad (12)$$

$$\alpha_{1,2} = \left(\frac{\sqrt{\gamma} \pm 1}{\gamma-1}\right)^2 \quad (\alpha_1 < \alpha_2).$$

Thus at $\alpha > \alpha_0$ system (II) is unstable in the neighbourhood of 0_1. Investigation of system (II) in infinity by means of the Poincaré transformations [36,37] shows that (II) has in a positive quadrant of infinity two more equilibrium states: 0_2-an infinite end of the x-axis, and 0_3-an infinite end of the y-axis (Fig. 2). The topology of these points is established by the theorem [37, p. 379]. According to this theorem, 0_3 in a positive quadrant consists of a parabolic sector and a part of a hyperbolic one at any $\gamma > 1$. The ω-separatrix of the saddle coincides with the equator of the Poincaré sphere, and α-separatrix approaches 0_3 in the direction coinciding with y-axis.

The topology of points 0_2 and 0_3 determined here can satisfy two types of the phase plane portrait. Whether one type or another holds true for system (II), both of them indicate the presence of a limit cycle in the case when point 0_1 is unstable (Fig. 2). This is confirmed by the numerical solution of system (II). Fig. 3 shows the transition of system (II) to the limit cycle at $\gamma = 2$, $\alpha = 1.1$. Numerical investigation of system (II) shows that the limit cycle increases with α, and becomes infinitely large at some finite α. However, it is to be noted that the transition of system (II) to an infinite limit cycle is in close relationship with an infinite increase of $\nu \cong \sigma_1\sigma_2^{\gamma}$, this being at variance with the condition $\nu_1 \ll 1$ by means of which system (5) was reduced to system (II). Hence the existence of an infinite limit cycle in system (II) has no meaning for system (5) and model (I).

The numerical analysis of system (5) shows that the limit cycle in this system is always bounded. Starting from this fact, it is not difficult to prove that the increase of $\varkappa_2$ from 0 to ∞ leads to an increase in the amplitude of oscillations which reaches its maximum and then drops again. It is possible that the dependence of the oscillation amplitude on $\varkappa_2$ has gaps, thus indicating the presence of hard self-excitation [38]. This question has not been investigated, however.

Thus, at

$$\alpha > \alpha_0 \tag{13}$$

in system (II) and hence in model (I) there appear self-oscillations. The frequency of self-oscillations is to the zero approximation given by

$$\omega_0 = \frac{1}{\sqrt{\gamma - 1}}. \tag{14}$$

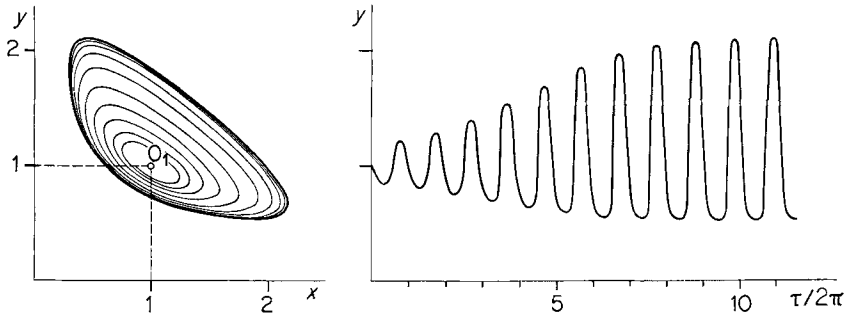

Fig. 3. *On the left: the transition of system (II) to the limit cycle from unstable focus 0_1; on the right: time display of the process.* The curves have been obtained by the digital computer solution of system (II) at a fixed integration step $h = 0.1$; $\gamma = 2$, $\alpha = 1.1$. x and y as in Fig. 2. τ is dimensionless time

When $\alpha \sim 1$ equation (14) can be used to calculate the frequency of self-oscillations with reasonable accuracy. For example, the frequency of self-oscillations at $\gamma = 2$ and $\alpha = 1.1$ calculated by the computer solution of system (II) proved to be equal to 0.967, whereas equation (14) predicts $\omega_0 = 1$. Thus in this case the error of calculation of the frequency by equation (14) amounts to 3.3%, this being quite satisfactory for zero approximation.

For greater convenience in comparing model (I) with the known experimental facts let us reduce expressions (13) and (14) by means of (6), (11), and (12) to the following form:

$$\frac{v_1}{k_2 K_2} < \left[\frac{(\gamma - 1)\, k_2 K_1}{V}\right]^{\frac{1}{\gamma}}; \tag{15}$$

$$T = 2\pi \left[\frac{K_1 K_2^{\gamma}}{v_1^{\gamma} V \sqrt{(\gamma - 1)^{\gamma - 1}}}\right]^{\frac{1}{\gamma + 1}} = \tag{16}$$

$$= \frac{2\pi}{k_2 \sqrt{\gamma - 1}} \tag{17}$$

where $K_1 \equiv (k_{-1} + k_{+2})/k_{+1}$; $K_2 \equiv \left(\frac{k_{-3}}{k_{+3}}\right)^{\frac{1}{\gamma}}$; $V = k_{+2}\, e_0$.

Expression (15) becomes the required condition for the appearance of self-oscillations in model (I). Expressions (16), (17) determine the period of self-oscillations.

As follows from (15), with γ held fixed the condition of self-excitation is satisfied more readily with lower v_1, and larger K_1, K_2, and k_2. The dependence of condition (15) on γ is more complicated. There exists an optimum value of γ,

$$\gamma_{\text{opt}} \cong 1 + \frac{V}{k_2 K_1} + \sqrt{\frac{2V}{k_2 K_1}\left(1 + \frac{V}{k_2 K_1}\right)} \tag{18}$$

when the right-hand side of inequality (15) is maximum. If γ drops from γ_{opt} to 1 or increases from γ_{opt} to ∞ the right-hand side of inequality (15) decreases

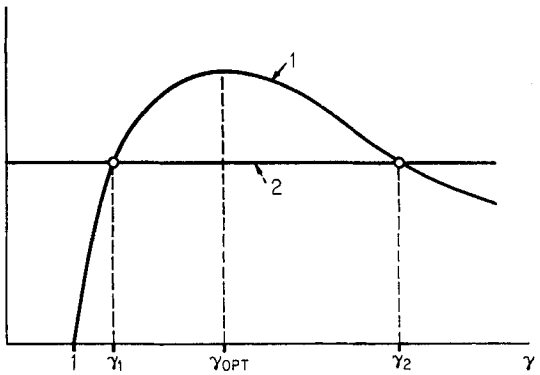

Fig. 4. *Graphic solution of inequality (15).* Curve 1 shows the dependence of the right-hand side of (15) on γ; a straight line 2 shows the dependence of the left-hand side of (15) on γ

to zero (Fig. 4). Hence it follows that inequality (15) can be satisfied only in a certain range of γ values, namely $\gamma_1 < \gamma < \gamma_2$, where $\gamma_{1,2}$ are positive roots of the equation

$$\frac{v_1}{k_2 K_2} - \left[\frac{k_2 K_1 (\gamma - 1)}{V}\right]^{\frac{1}{\gamma}} = 0. \tag{19}$$

Thus a certain moderate degree of substrate inhibition determined by the range (γ_1, γ_2) is favourable to the appearance of self-oscillations in model (I). An excessive $(1 < \gamma < \gamma_1)$ or, conversely, too weak substrate inhibition $(\gamma > \gamma_2)$ results in damping self-oscillations.

Fig. 4 shows that a decrease in the ratio $v_1/k_2 K_2$ leads to a widening of the interval on which in-

equality (15) is satisfied. Consequently, the range of γ values corresponding to the self-oscillatory state in model (I) increases with a decrease in the source rate v_1.

In the glycolytic extracts showing self-oscillations among the enzymes of the Embden-Meyerhof pathway pyruvate kinase and phosphofructokinase have the lowest activity [5—9,36]. Hence it follows that in such extracts the sink of fructose-1,6-diphosphate is controlled by pyruvate kinase which is very far from being saturated because of an extremely low level of the glycolytic flux. This makes it possible to think that the sink rate constant k_2 in (15,17—19) is proportional to V_{PK}/K_{PK} where V_{PK} is the maximum rate of the pyruvate kinase reaction, and K_{PK} is the Michaelis constant for phosphoenolpyruvate.

DISCUSSION

The above analysis leads to an important conclusion that the experimental study of self-oscillations in the phosphofructokinase reaction may be considerably simplified. In fact, according to model (I) all the glycolytic reactions, except for the phosphofructokinase reaction, are not essential for the appearance of self-oscillations, their function being to supply the substrate at the necessary rate and to remove the products. This implies that under experimental conditions the part of the glycolytic system feeding substrates to the phosphofructokinase reaction may be replaced by the capillaries through which substrate solutions are continuously delivered into a spectrophotometric cell. From the second half of the glycolytic system there may be taken only the part which ensures an irreversible sink of the products and affords a spectrophotometric recording of the process. Thus in studying glycolytic self-oscillations it is possible to do without using extracts and to replace them by a reconstituted system containing a small number of purified enzymes.

Condition (15) shows that self-oscillations in reaction (1) arise more readily with lower enzyme-product affinity $\left(\frac{1}{K_2}\right)$. In this respect reaction (I) is similar to a product-inhibited reaction [37,39]. The resemblance of these two types of reactions is strengthened by the fact that both of them require a marked substrate inhibition of the enzyme for the self-oscillatory state to be realized. In the absence of substrate inhibition only damped oscillations may exist in such reactions.

This conclusion is at variance with the assertion of Higgins [12,13] about the possibility of self-oscillations in the phosphofructokinase reaction in the presence of product activation alone ($\gamma = 1$). Higgins came to this conclusion on the basis of the model in which all the constants were chosen arbitrarily [12, 13]. However, for the model [12,13] to comply with the known experimental facts [9,24—30] the enzyme concentrations in this model must be far smaller than substrate and product concentrations, and all the normalized rate constants [see (2)] must be well over 1. Besides, account must be taken of the fact that both the phosphofructokinase reaction and the sinks of its products are far from being saturated since in a self-oscillatory state the rate of the glycolytic flux is much lower than the maximum rate of the phosphofructokinase reaction and that of the reactions controlling the sink of fructose-1,6-diphosphate and ADP [6—10]. With regard to this the model of Higgins takes the form [39,40]:

$$\left.\begin{aligned} \frac{d\sigma_1}{d\theta} &= \nu_1 - \frac{\sigma_1\sigma_2}{1+\sigma_2(1+\sigma_1)} \\ \frac{d\sigma_2}{d\theta} &= \alpha_2\left[\frac{\sigma_1\sigma_2}{1+\sigma_2(1+\sigma_1)} - \frac{\nu_2\sigma_2}{\varkappa_2}\right] . \end{aligned}\right\} \quad (20)$$

According to the Dulac-Bendixson criterion [41] system (20) has no limit cycle in a positive quadrant of the phase plane with all the positive values of its parameters [40]. At $\nu_1 \ll 1$ system (20) is reduced to the Lotka model [35] with one autocatalysis which is not known to be self-oscillatory.

Thus the Higgins' system has no limit cycle with those parameter values with which self-oscillations in glycolysis are observed. However, the model of Higgins, as it has been shown in [40], has a limit cycle when the sink of the product is saturated. In this case the model of Higgins takes the form:

$$\left.\begin{aligned} \frac{d\sigma_1}{d\theta} &= \nu_1 - \frac{\sigma_1\sigma_2}{1+\sigma_2(1+\sigma_1)} \\ \frac{d\sigma_2}{d\theta} &= \alpha_2\left[\frac{\sigma_1\sigma_2}{1+\sigma_2(1+\sigma_1)} - \frac{\nu_2\sigma_2}{\varkappa_2+\sigma_2}\right] \end{aligned}\right\} \quad (21)$$

where ν_2 is the relative maximum rate of the sink and $\varkappa_2$ is the relative Michaelis constant for the sink. Other notations coincide with those in (6) at $\gamma = 1$.

In system (21) at

$$\alpha_2 > \frac{\nu_2\varkappa_2^2(1-\nu_1)^2}{(\nu_2-\nu_1)^2(1-\varkappa_2-\nu_2)} \quad (22)$$

there appear self-oscillations (soft excitation [38]) with the frequency which in a linear approximation is given by

$$\omega_0 = \varkappa_2(1-\nu_1)^2\sqrt{\frac{\nu_1}{(1-\varkappa_2-\nu_2)(\nu_2-\nu_1+\nu_1\varkappa_2)}} .$$

As seen from (22), self-oscillations in (21) can exist only in the case when

$$\nu_1 < \nu_2 < 1-\varkappa_2. \quad (23)$$

Condition (23) and, probably, condition (22) can be secured in a reconstituted glycolytic system.

The next point to be discussed is the compliance of model (I) with the experimental data.

The appearance of sustained oscillations in cell extracts on addition of some polysaccharides is

probably the most striking fact discovered by Pye [2,3] and confirmed afterwards by Pye and Chance [4], Hess *et al.* [5—9] and Frenkel [11]. This fact which has no satisfactory explanation thus far, can be easily understood in terms of model (I).

Actually, an addition to the extracts of trehalose [2—9] and glycogen [11] instead of glucose brings about a sharp decrease in the rate of formation of fructose-6-phosphate due to the lower activity of trehalase [5,7—9] and probably, α-glucanphosphorylase in comparison with the activity of hexokinase. But such a decrease in the rate of formation of fructose-6-phosphate, equivalent to the decrease in the source rate v_1 in model (I), favours production of self-oscillations as follows from (15).

Frenkel [11] found an original way of limiting the glycolytic flux on the phosphofructokinase reaction step by adding apyrase to the extract. This resulted in a decrease in the ATP flux into the phosphofructokinase reaction. A decrease in the ATP flux as well as in the fructose-6-phosphate flux is equivalent to a decrease in the source rate v_1 in model (I). So it is quite natural (see 15,16) that an addition of apyrase leads to the initiation of self-oscillations and at the same time to an increase in the self-oscillation period as compared to that in the control experiment.

The decrease of γ corresponding to increasing substrate inhibition causes an increase in the period of self-oscillations (see 16,17). This property of the model is in good agreement with the data of Frenkel [11] who has shown that the successive additions of ATP to the extracts increase the period of oscillations. The descrease in a damping factor observed in the case indicates (Fig.4) that the initial degree of substrate inhibition in the phosphofructokinase reaction was not large ($\gamma > \gamma_2$).

It has been found that the amplitude and period of self-oscillations vary inversely with the pyruvate kinase concentration [42] as well as with the concentration of fructose-1,6-diphosphate [19,42—45] which was shown to be a strong activator of pyruvate kinase [46—48]. This again is in good agreement with the model. Indeed, it follows from equation (17), an increase in the pyruvate kinase activity (an increase in k_2) results in a decrease in the period of self-oscillations. At the same time the steady state amplitude of oscillations decreases if k_2 is sufficiently large.

Condition (15) is satisfied more readily with a large k_2. This is in agreement with the data of Hess and Brand [43] who found that an increase in k_2 on addition of phosphoglyceratekinase to the extract led to a decrease in damping factor as well as in the period and amplitude of damped oscillations.

Unfortunately, we cannot compare the model and the phosphofructokinase reaction under conditions of varying concentrations of different deinhibitors such as AMP, 3′,5′-cyclic AMP, *etc.*, since these substances change almost all the parameters of the phosphofructokinase reaction. In order to follow in the model the changes induced by addition of some deinhibitors a knowledge of the relationship between the parameters γ, K_1, K_2, V and the concentration of a deinhibitor is necessary. Such data are not available as yet.

In conclusion it should be noted that the suggested model is the simplest. It results from severe simplifications and limitations imposed on the actual glycolytic system. Some known experimental facts such as the phenomenon of double-frequency self-oscillations [4,8—10], reversibility of the substrate sources [49], possibility of several alternative steady states [50—53] and some other things were deliberately left out of consideration. Therefore, the model should be considered as a first approach to a close understanding of the actual oscillatory mechanism.

It is remarkable, however, that in spite of all these simplifications the model describes qualitatively the basic dynamic properties of the phosphofructokinase reaction correctly. This implies that the main variables of self-oscillatory glycolytic mechanism have been correctly chosen. This may be quite strictly proved by the corresponding mathematical theorems which are generalizations of the Tikhonov's theorem used above. In subsequent papers [54,55] it will be shown that model (5) can be obtained from the full scheme of the glycolytic system by means of limit transitions eliminating fast variables.

It is to be hoped that the model can be used for a quantitative description of glycolytic self-oscillations as well. For this purpose, however, it is necessary to have numerical data from the same source.

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