INTERACTION OF EMBDEN–MEYERHOF PATHWAY
AND HEXOSE MONOPHOSPHATE SHUNT IN
ERYTHROCYTES

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A mathematical model of glycolysis in human erythrocytes describing the functioning of the Embden–Meyerhof and pentose phosphate pathways based on their interaction has been developed. The characteristic surfaces, i.e., the dependences of the rates of metabolite flows in both pathways on the ATP and NADPH concentrations, were calculated. The model is in good accord with the experimental data on the characteristics of glycolysis at low flow rates through the pentose phosphate pathway. It follows from the model that the NADRH and GSH concentrations should be stabilized. In the range of physiological ATP and NADPH concentrations the Embden–Meyerhof and pentose phosphate pathways function almost independently. When the NADPH concentration becomes lower than 80% of the physiological value, the system ceases to stabilize the ATP concentration. In turn, a decrease in the ATP concentration leads to a proportional decrease in the maximum rate of the pentose phosphate pathway.

The hexose monophosphate shunt plays an important role in erythrocyte metabolism. Protection of the cell against destructive oxidative processes is associated with this pathway [1–3]. The pentose phosphate pathway (PPP) is closely linked with the Embden–Meyerhof pathway (EMP). The two systems have a number of enzymatic steps in common. Hexokinase is the first enzyme to the two systems and limits the overall flow rate [4]. Normally the flow through the PPP does not exceed 10% of the total flow of glucose [1–3]. However, intensification of oxidative processes can lead to an increase of tens of times in this fraction [2, 3]. In this case almost all of the glucose is consumed in the PPP. Naturally, this has a sharp effect on the state of the Embden–Meyerhof pathway.

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There have been a large number of experimental studies of the regulation of the EMP [4-7]. There are several mathematical models that describe the regulation of this system fairly well [8-11], however, they assume that the flow of metabolites through the PPP is equal to zero.

A great deal of experimental data has been obtained concerning the regulation of the PPP [1-4]. In the only theoretical study [12] an attempt was made to generalize these results. The mathematical model constructed in it correctly describes a number of important features in the functioning of the PPP, however, it does not take into account the regulation of the EMP, which makes it unsuitable for an analysis of a whole series of experimental situations.

In the present work for an investigation of the interaction of the EMP and PPP a mathematical model incorporating both systems was developed. It is based on a model of the EMP giving a good quantitative description of this pathway at a zero rate of oxidative processes [10, 11] and the model of the PPP from [12].

It was shown in [7] that the dependence of the rate of ATP production on the ATP concentration (a characteristic of the EMP) is a very informative characteristic of the functioning of the ATP–producing system. Analogously, the dependence of the rate of production of reducing equivalents on the NADPH concentration (a characteristic of the PPP) is an important property of the functioning of the pentose phosphate pathway. In a complete system the steady-state flows of metabolites in the two pathways depend on the concentrations of both ATP and NADPH. Therefore, it is convenient to describe the behavior of the system as a whole by the characteristics surfaces; i.e., by the dependences of the rates of ATP and NADPH production on two variables: the concentrations of these compounds. The construction of characteristic surfaces using a mathematical model was the objective of this work.

RESULTS

Mathematical Model. It was shown in [10] that the flow through the Embden–Meyerhof pathway is determined by the first three enzymes of glycolysis: hexokinase (EC 2.7.1.1), phosphohexoisomerase (EC 5.3.1.9), and phosphofructokinase (EC 2.7.1.11). An analysis of the experimental data on the regulation of the PPP and of the data of [12] show that the flow through the shunt is determined by the first enzyme of hexosemonophosphate shunt: glucose-6-phosphate dehydrogenase (EC 1.1.1.49) [1, 3]. Data on the steady-state kinetics of these four enzymes and the stoichiometry of the two systems under steady-state conditions are the basis of the model.

The following notation will be used: \( x_1 \) is the glucose-6-phosphate concentration; \( x_2 \) is the fructose-6-phosphate concentration; \( x_3 \) is the fructose-1,6-diphosphate concentration; \( x_4 \) is the glyceraldehyde phosphate concentration; \( y_1 \) is the NADP concentration; \( y_2 \) is the NADP concentration; \( y_3 \) is the GSH concentration; \( z_1 \) is the ATP concentration; \( z_2 \) is the ADP concentration; \( z_3 \) is the AMP concentration; \( u_1 \) is the rate of ATP-consuming reactions; \( u_2 \) is the rate of the hexokinase-reaction; \( u_3 \) is the rate of the phosphofructokinase reaction; \( u_4 \) is the rate of the glucose-6-phosphate dehydrogenase reaction (the rate of the PPP); \( u_5 \) is the rate of lactate production; \( u_6 \) is the rate of NADP oxidation.

According to the scheme depicted in Fig. 1, glucose is converted by hexokinase to glucose-6-phosphate at rate \( u_1 \), this flow then bifurcates. Part of the glucose-6-phosphate is converted to fructose-6-phosphate at rate \( u_2 \) and the latter is then converted with the aid of phosphofructokinase to 1,6-fructose diphosphate at rate \( u_3 \). Rates \( u_2 \) and \( u_3 \) are not equal to each other, since fructose-6-phosphate is produced in the shunt at a rate of \( \frac{1}{2} u_4 \). In the given model the steady-state flow through the shunt is determined by the rate of the glucose-6-phosphate dehydrogenase reaction. Carbon dioxide is formed at the same rate, while NADP is reduced at a rate of 2 \( u_4 \). In addition to fructose-6-phosphate, glyceraldehyde-3-phosphate is produced in the PPP at a rate of \( \frac{1}{2} u_4 \). Glyceraldehyde-3-phosphate is also produced in the EMP and is the site of the fusion of the two flows. The subsequent transformations of glyceraldehyde-3-phosphate take place at rate \( u_5 \) and are common to both systems. In the process two ATP molecules are formed from each glyceraldehyde-3-phosphate molecule at a rate of 2 \( u_5 \). ATP is consumed in the hexokinase and phosphofructokinase reactions at rates \( u_1 \) and \( u_2 \), respectively. The complete system of equations and rates in the PPP and EMP takes the following form:

\[
\begin{align*}
\dot{x}_1 &= u_1 - u_2 - u_3 = 0, \\
\dot{x}_2 &= u_2 - u_3 - \frac{1}{2} u_4 = 0, \\
\dot{x}_3 &= 2 u_3 - u_4 - u_5 = 0, \\
\dot{y}_1 &= 2 u_4 - u_5 = 0, \\
\dot{z}_1 &= 2 u_5 - u_6 - u_7 = 0.
\end{align*}
\]
Fig. 1. Scheme of flows in Embden–Meyerhof and pentose phosphate pathways used in constructing mathematical model (notation and explanations in text).

\[ y_i + y_i = n = \text{const}, \]  
\[ z_i + z_3 + z_3 = a = \text{const}, \]  
\[ \frac{x_3}{x_2} = 0.5. \]  

(6) \hspace{1cm} (7) \hspace{1cm} (8)

Here, as in [10], it is assumed that the adenylate kinase reaction is close to equilibrium, while the pool of adenylates remains constant; the pool of NADP is also assumed to be constant.

The rate of the hexokinase reaction takes the form [11]:

\[ u_1 (x_2, x_3) = \alpha_1 \frac{x_1}{k_{11} + k_{11} x_2 k_{11} x_3}, \]

where \( k_{11} = 10^{-3} \text{ M}; \ k_{12} = 1.2 \cdot 10^{-5} \text{ M}; \ \alpha_1 = 1.2 \cdot 10^{-2} \text{ M/h} \). The reaction is assumed to be irreversible, it depends on the ATP concentration, and is inhibited by glucose-6-phosphate.

The rate of the phosphohexoisomerase reaction is described by the equation [10]:

\[ u_2 (x_2, x_3) = \alpha_2 \frac{x_4}{k_{21} + k_{22} x_2 + k_{23} x_3}, \]

where \( k_{21} = 3; \ k_{22} = 3 \cdot 10^{-4} \text{ M}; \ k_{23} = 2 \cdot 10^{-4} \text{ M}; \ \alpha_2 = 3.6 \cdot 10^{-1} \text{ M/h}. \)

The equation for the rate of the phosphofructokinase reaction takes the form [11]:

\[ u_2 (x_3, x_2, x_3) = \alpha_3 \frac{1}{(1 + x_3 + x_2) (1 + x_3 + x_2)} \left[ \frac{1 + x_3 + x_2}{k_{21} + k_{22} x_2 + k_{23} x_3} \right], \]

where \( \alpha_3 = 0.4 \text{ M/h}; \ k_{21} = 10^{-4} \text{ M}; \ k_{22} = 2 \cdot 10^{-3} \text{ M}; \ k_{23} = 10^{-5} \text{ M}; \ k_{24} = 10^{-4} \text{ M}; \ k_{25} = 0.37 \cdot 10^{-5} \text{ M}. \)

The glucose-6-phosphate dehydrogenase reaction is assumed to be irreversible; its rate depends on the concentrations of the substrates: glucose-6-phosphate and NADP and it is inhibited by NADPH [12]:

\[ u_4 (x_2, x_3, x_4) = A \frac{x_2 x_3}{(1 + x_4)} \]

where \( A = 2; \ L = 10^8; \ \alpha_3 = 0.4 \text{ M/h}; \ k_{32} = 10^{-4} \text{ M}; \ k_{33} = 2 \cdot 10^{-3} \text{ M}; \ k_{34} = 10^{-5} \text{ M}; \ k_{35} = 10^{-4} \text{ M}; \ k_{36} = 0.37 \cdot 10^{-5} \text{ M}. \)
Fig. 2. Dependence of rate of ATP production on ATP and NADPH concentrations. Characteristic surface of $u(y, z)$:

$$y = \frac{[NADPH]}{[ATP] + [ADP] + [AMP]}$$

$$z = \frac{[ATP]}{[ATP] + [ADP] + [AMP]}$$

where $k_{11} = 4 \cdot 10^{-5}$ M; $k_{12} = 4 \cdot 10^{-6}$ M; $k_{13} = 2 \cdot 10^{-5}$ M; $\alpha_4 = 2.5 \cdot 10^{-2}$ M/h; $n = 5 \cdot 10^{-5}$ M.

Construction of the characteristic surfaces $u_1(y, z), u_2(y, z), u_3(y, z), u_4(y, z)$ is the goal of this work. Here $y = \frac{y_2}{n}, z = \frac{z_1}{a}$. Since in constructing the characteristic surfaces of ATP and NADPH concentrations are the parameters, Eqs. (4) and (5) are omitted from the system.

The dependence of the rate of ATP production on $z$ and $y$ is depicted in Fig. 2. The surface is saddle-shaped. The sections of this surface intercepted by the planes $y = \text{const at } y \text{ close to } 1$ take the form of bell-shaped curves. At $y = 1$ the section coincides with the characteristic curve of glycolysis obtained in the model of the EMP [11]. As $y$ decreases the descending segment of the characteristic curve of the EMP in the vicinity of the physiological ATP concentration becomes flatter and flatter and at $y \to 0$ it is replaced by an ascending segment.

The dependence of the rate of glucose consumption on the variables $y$ and $z$ is depicted in Fig. 3. In this case a decrease in $y$ also leads to a rapid increase in the rate and to the disappearance of the descending segment.

The surfaces $u_2(y, z)$ and $u_4(y, z)$ are depicted in Figs. 4 and 5, respectively. At $y = 1$ the sections of the surfaces $u_2(y, z), u_4(y, z)$ and $u_5(y, z)$ coincide. The relation $u_4(z)$ does not change qualitatively with changes in $y$. A decrease in $y$ leads to a considerable decrease in $u_4$, especially at $z > 0.7$. The rate $u_4$ (Fig. 5) increases monotonically with an increase in $ATP$ and a decrease in NADPH.

The concentration of glucose-6-phosphate, the key metabolite situated at the branching point of the flows, increases slightly with an increase in $z$ and is low everywhere except in the vicinity of the point $(z = 1, y = 1)$ (Fig. 6), where it increases very rapidly.

So far we have not considered the metabolism of glutathione, which is the main reducing agent in erythrocytes. Glutathione is reduced in erythrocytes in only one reaction, the glutathione reductase reaction [1]. NADPH is the reducing agent in this reaction. The glutathione reductase activity is very high in normal erythrocytes [3]. For all practical purposes this reaction can be used to be an equilibrium reaction [3, 12]. This means that the glutathione and NADPH concentrations are clearly linked by the simple relation

$$\frac{2k_2 z^2}{1-z} \cdot \frac{1-y}{y} = k_s.$$
Fig. 3. Dependence of rate of glucose consumption on ATP and NADPH concentrations.

Fig. 4. Dependence of flow through phosphofructokinase step on ATP and NADPH concentrations.

Fig. 5. Dependence of flow through pentose phosphate pathway on ATP and NADPH concentrations.
where \( s = s_2 / (2s_1 + s_2) \); \( 2s_1 + s_2 = s_3 = \text{const} \); \( s \) is the relative concentration of reduced glutathione; \( s_1 \) and \( s_2 \) are the concentrations of the oxidized and reduced forms of glutathione, \( k_3 \) is the equilibrium constant of the glutathione reductase reaction, and \( k_3 = 100 \text{ mM} \) [12].

Using Eq. (9), the dependence of the rate of the PPP on the glutathione concentration \( u_4(s, z) \) can be obtained, which qualitatively takes the same form as the relation \( u_4(y, z) \). The sections of the surface \( u_4(s, z) \) intersected by the planes \( z = \text{const} \) are depicted in Fig. 7a. It is seen that the rate of the PPP shows a very slight dependence on \( s \) in almost the whole range of concentrations and the rate drops sharply only at \( s > 0.9 \), approaching zero at \( s = 1 \).

DISCUSSION OF RESULTS

Let us examine the behavior of the system as a whole in the vicinity of physiological ATP and NADPH concentrations. We shall denote the physiological ATP concentration in relative values of \( z_0 \) and the relative value of the physiological NADPH concentration by \( y_0 \).

It is known from the literature data that under physiological conditions \( z_0 = 0.85 \); \( y_0 = 0.9 \) [2, 5, 7]. At physiological values of \( y \) and \( z \) almost the entire flow of glucose goes through the EMP, the rate \( u_4 \) is low, and the value of \( x_4 \) is about 100 \( \mu \text{M} \). At a slight decrease in the ATP concentration (i.e., at an increase in the rate of ATP-consuming processes) the value of \( x_4 \) drops sharply, the rate of glucose consumption and ATP production increases, while the rate \( u_4 \) in the value of \( y \) remains almost unchanged. At a slight decrease in \( y \) (intensification of oxidative processes) the value of \( x_4 \) and rate \( u_4 \) drop, rates \( u_4 \) and \( u_4 \) increase, and \( u_4 \) changes slightly, i.e., the proportion of glucose utilized in the PPP, which goes into the reduction of NADP, increases, while the rate of ATP production remains almost unchanged.

Thus, at slight deviations in the ATP and NADPH concentrations from the physiological values the EMP and PPP function independently as ATP- and NADPH-producing systems.

At sharper changes in \( z \) and \( y \) the EMP and PPP do not function independently. When \( y \) drops below 80% of the physiological value, the descending segment on the surface \( u_4(y, z) \) disappears (Fig. 7b) and the ATP-producing system ceases to stabilize the ATP concentration.

A decrease in \( z \) leads to a proportional decrease in \( u_4 \), i.e., the ability of the erythrocytes to withstand oxidative processes drops.

Under physiological conditions almost all of the glutathione is in a reduced state, the rate of the PPP is low [13], and, as Fig. 7a shows, a slight change in the GSH concentration leads to a considerable increase in the rate of its reduction, which provides stabilization of the GSH level in the erythrocytes. The coefficient of the stabilization of NADPH is considerably lower; this is due to the higher value of the equilibrium constant of the glutathione reductase reaction, which is shifted in the direction of GSH production.
The decrease in the maximum rate of the PPP, which is proportional to the drop in the steady-state ATP concentration (Fig. 7a), is most important. Thus, the model shows that the threshold of oxidative hemolysis of the erythrocytes should be proportional to the level of ATP.

LITERATURE CITED


INTERACTION OF MEMBRANE TRANSPORT

PROTEINS IN E. coli K12


The kinetics of the inhibitory effect of MeGlc on the transport of NO₂-PheGal in E. coli K12 was studied. The indicated effect was found only at a specific quantitative ratio of the protein carriers responsible for the transport of these substances: enzyme II[Gal] and β-d-galactoside permease. It was found that at this ratio not only is MeGlc capable of inhibiting the transport of β-galactosides, but β-galactosides (GalSGal) can decrease the rate of accumulation of MeGlc. Data indicating that the conformation of the two membrane proteins changes in the region of the maximum inhibitory effect, which leads to intensification of the activity of enzyme II[Gal] and to an increase in its affinity for MeGlc, were obtained. It is believed that this phenomenon is not unique, but reflects the general principle that under certain conditions many bacterial membrane proteins can interact, changing their activity.

The phosphoenolpyruvate-dependent carbohydrate-phosphotransferase system consists of membrane (enzyme II) and cytoplasmic (enzyme I, protein Hpr, factor III) components. The main function of this system is the transmembrane transport of carbohydrates and hexahydrated alcohols, coupled with phosphorylation, in bacteria of different species [1]. Glucose and its nonutilizable analog MeGlc are transported in E. coli by the glucose-phosphotransferase system according to the following scheme:

\[
\text{PEP} + \text{Hpr} \xrightarrow{\text{enzyme I}} \text{MeGlc} + \text{Hpr} + \text{pyruvate.}
\]

The last reaction proceeds with the participation of the protein pair II[Gal]/III[Gal] (enzyme II[Gal] for short), specific for glucose and MeGlc; II[Gal] is an integral membrane protein. Glucose can also penetrate into cells with the participation of the integral membrane proteins IIa/IIb (enzyme II[man] for short), specific for mannose and Dglu.

The PTS is not only involved in the transport of carbohydrates; it is also required for regulation of the activity of permeases, which carry out the transport of a number of different substrates [1]. Thus, even before the discovery of the PTS it was known that both glucose and MeGlc are capable of inhibiting the transport of β-galactosides, which is accomplished with the participation of an intrinsic permease (the product of gene lac Y [2]). This phenomenon was subsequently studied in detail by Winkler and Wilson [3], as well as in a series of studies.

Abbreviations used: PTS) Phosphoenolpyruvate-dependent carbohydrate-phosphotransferase system; enzyme II[Gal] membrane component of PTS specific for glucose and MeGlc (EC 2.7.1.69); enzyme II[man] membrane component of PTS specific for mannose and Dglu (EC 2.7.3.9); Hpr) thermostable, low-molecular-weight protein, component of PTS; M-protein β-galactoside permease, product of gene lac Y; MeGlc methyl-α-D-glucopyranoside; MeGlcP methyl-α-D-glucopyranosyl-6-phosphate; MalNEm) N-ethylmaleimide, Dglu 2-deoxy-D-glucose; NO₂-PheGal α-nitrophenyl-β-D-galactopyranoside; SMeGal) methylthio-β-D-galactopyranoside; PropSGal) isopropylthio-β-D-galactopyranoside; GalSGal) galactosylthio-β-D-galactopyranoside.

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