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Deficiencies of glycolytic enzymes as a possible cause of hemolytic anemia

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Abstract

The critical minimum values of Na,K-ATPase and glycolytic enzyme activities at which the erythrocyte viability is lost were calculated using the mathematical model of the erythrocyte, which included all reactions of glycolysis, adenylate metabolism, ionic balance, and osmotic regulation of erythrocyte volume. The criterion for cell death was an increase in its volume to the level at which it is sequestrated from the circulation or is lysed. In hemolytic anemia associated with hexokinase or pyruvate kinase deficiency, activities of these enzymes measured in patient erythrocytes appeared to be close to the calculated critical values. By contrast, in hemolytic anemia associated with phosphofructokinase, glucosephosphate isomerase, triosephosphate isomerase, or phosphoglycerate kinase deficiency, activities of these enzymes measured in patient erythrocytes were significantly greater than the calculated critical values. In this case, if the deficient enzyme were stable, i.e. its activity in the cell were low, but constant in time, the deficiency observed would not account for the erythrocyte destruction observed and the development of hemolytic anemia. It was shown, however, that in phosphofructokinase, glucosephosphate isomerase, triosephosphate isomerase, or phosphoglycerate kinase deficiency, hemolytic anemia can arise because of the instability of these enzymes in time.

Keywords: Erythrocyte; Glycolysis; Enzyme deficiency; Hemolytic anemia; Mathematical model

1. Introduction

In many patients with hemolytic anemia, a deficiency of one of glycolytic enzymes is found [1–4]. Usually, it is assumed that the abnormal enzyme with decreased activity slows down the glycolytic flux, preventing cells from producing energy at the rate necessary to maintain their viability and thereby causing erythrocyte destruction and anemia. However, a common finding in hemolytic anemia is a 5–20-fold decrease in the activities of the so-called non-key enzymes (including GPI, TPI, and PGK) whose normal activities are about 1000-fold higher than the rate of glycolytic flux. Obviously, their activities are still higher by 50–200-fold than the rate of glycolytic flux and cannot be expected to affect it seriously. Moreover, anemia severity does not often correlate with decreased enzymatic activities. Therefore, a question arises of whether the deficiency observed is actually the cause of anemia.

Earlier, the effects of glycolytic enzyme deficiency on the viability of erythrocytes have been studied by means of mathematical modeling [5,6]. However, the criteria for cell death used in these models were somewhat arbitrary. They included a considerable decrease in ATP and appreciable accumulation of glycolytic intermediates. Analysis of these models showed that the activities of HK, PK, and, in some cases, PFK and PGK decreased to the level observed in patients with hemolytic anemia are insufficient to maintain the erythrocyte viability. On the other hand, the

Abbreviations: HK, hexokinase; GPI, glucosephosphate isomerase; PFK, phosphofructokinase; ALD, aldolase; TPI, triosephosphate isomerase; GAPDH, glyceraldehyde phosphate dehydrogenase; PGK, phosphoglycerate kinase; DPGM, diphosphoglycerate mutase; DPGP, diphosphoglycerate phosphatase; PGM, phosphoglycerate mutase; ENO, enolase; PK, pyruvate kinase; LDH, lactate dehydrogenase; AMPD, AMP deaminase; AMPP, AMP phosphatase; AK, adenylate kinase; GLU, glucose; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FDP, fructose-1,6-diphosphate; DAP, dihydroxyacetone phosphate; GAP, glyceraldehyde-3-phosphate; 1,3-DPG, 1,3-diphosphoglycerate; 2,3-DPG, 2,3-diphosphoglycerate; 3-PG, 3-phosphoglycerate; 2-PG, 2-phosphoglycerate; PEP, phosphoenolpyruvate; PYR, pyruvate; LAC, lactate; PO4, orthophosphate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; NAD, nicotinamide adenine dinucleotide oxidized; NADH, nicotinamide adenine dinucleotide reduced

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values of GPI, TPI, and, in certain cases, PGK activities in patients appeared much higher than the model-predicted minimum at which cells lose their viability (1% of the normal activity or less). Hence, a decrease in activities of these enzymes per se cannot be the cause of erythrocyte destruction observed in anemia. However, the discrepancy between the calculated permissible minimum values of activities of non-key enzymes and the values determined experimentally in anemia can be eliminated by the assumption that the deficient enzyme is unstable and its activity varies with time taking values from normal ones to the permissible minimum [5].

This study is an attempt to estimate the permissible minima (critical values) of glycolytic enzyme activities and activity of Na,K-ATPase in erythrocytes using a model describing all reactions of glycolysis, adenylate metabolism, ionic balance, and osmotic regulation of the cell volume. An increase in cell volume to some critical value was considered to be the natural criterion for erythrocyte destruction. Normal erythrocytes are discoid and, hence, their surface area-to-volume ratio is high. The discoid shape of erythrocytes ensures optimal rheological characteristics of these cells. As the erythrocyte membrane is inextensible, its shape must be maintained by mechanisms stabilizing the cell volume. The erythrocyte volume is kept almost constant owing to operation of transport Na,K-ATPase, which utilizes energy produced in glycolysis. Obviously, if transport Na,K-ATPase and/or glycolytic enzymes are abnormal, the volume and, hence, rheological characteristics of erythrocytes will be also abnormal. This results in their sequestration from the circulation or even osmotic lysis. In the population of circulating erythrocytes, the maximum deviation of the surface area-to-volume ratio from its mean is less that 10% [7–9]. It is likely that the cells with greater deviations are sequestrated from the circulation. Therefore, one criterion for cell destruction was chosen to be a 10%-increase in the cell volume. The second criterion for erythrocyte death was an increase in its volume by 50% of the normal value, which results in erythrocyte lysis [8,9].

In general, metabolic impairments and cell death may result from any significant change (whether increase or decrease) in the activity of a particular enzyme. Therefore, we analyzed in the model the responses of the erythrocyte to increases, as well as decreases, in enzymatic activities.

A preliminary account of some results of this study has been published earlier [10,11].

2. Mathematical model

The model was constructed to describe erythrocyte energy metabolism (including ATP production in glycolysis (Fig. 1) and ATP consumption by Na,K-ATPase and in other processes), adenylate metabolisms, ion transport through the cell membrane, and erythrocyte volume, which was determined from osmotic balance between the cell and its environment. The mathematical model and methods of its investigation are described in Appendix A.

3. Results and discussion

The stationary intracellular concentrations of metabolites, ions, and their fluxes calculated using the model for the normal physiological values of parameters are shown in Table 1. These data are well consistent with the results of experimental studies of human erythrocytes.

Analysis of the model showed that if the activity of any of the glycolytic enzymes (except DPGM) drops to a certain minimum, the erythrocyte volume increases until the cell lyses. The volume increases because either the steady state is lost when glycolysis becomes unable to power active transport of ions (in HK, GPI, PFK, and ALD deficiencies) or the stationary concentrations of osmotically active metabolites of glycolysis increase (in TPI, PGK, DPGP, PGM, ENO, and PK deficiencies). In GAPDH, and LDH deficiencies, a 10% increase in the volume results from accumulation of glycolytic intermediates, whereas a 50% increase is a consequence of the loss of the steady state. If DPGM activity diminishes to zero,

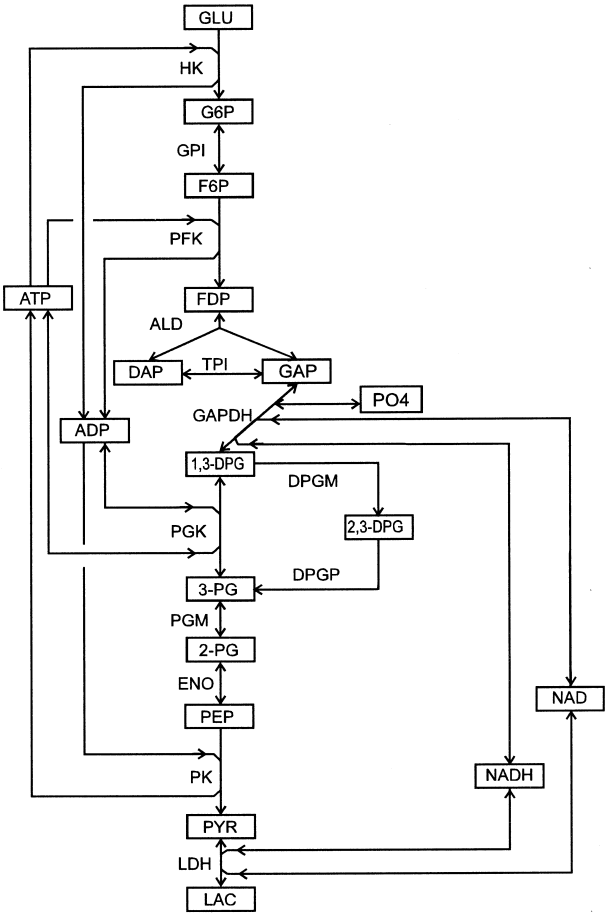

Fig. 1. Schematic presentation of glycolysis in human erythrocytes.

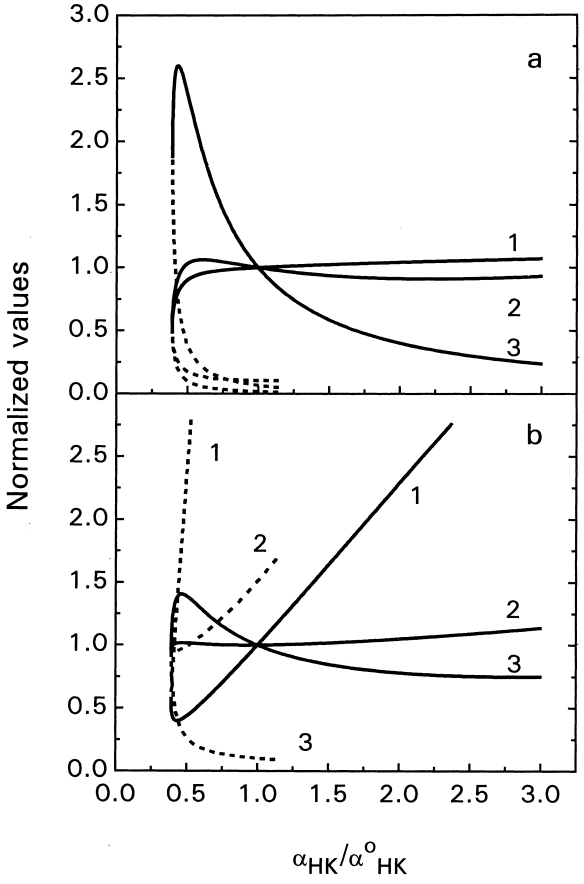

Fig. 2. Dependence of the stationary values of the model variables on the HK activity. Solid and dashed lines correspond to the stable and unstable states. Stability/instability of the stationary state is considered within the framework of the theory of dynamical systems [26,27]. A stationary state is said to be stable if the cell experiencing small fluctuations tends to return to this state and remains in this state or close to it for an infinitely long time. Conversely, if the cell experiencing small fluctuations tends to deviate farther from the stationary state, this stationary state is called unstable. (a) 1, G6P; 2, 2,3-DPG; and 3, ATP. (b) 1, intracellular Na; 2, erythrocyte volume; and 3, the total concentration of osmotically active metabolites. Here and thereafter, α denotes enzymatic activity; the physiological (normal) values of the parameters and variables are marked by '0'.

the erythrocyte metabolism and volume almost do not change.

The results of simulation showing how HK activity affects the stationary intracellular metabolite concentrations, Na ion concentration, and erythrocyte volume are presented in Fig. 2. With decreasing HK activity, the stationary ATP concentration first increases, but then drops abruptly (Fig. 2a). In the model, an increase in the ATP concentration with decreasing HK activity results from the increase in the adenylate pool. In this way, adenylate metabolism compensates for the decrease in HK activity. This compensation allows the erythrocyte to stabilize its volume over changes in cell parameters [28,29]. An increase in the ATP concentration is accompanied by a decrease in the intracellular Na concentration (owing to acceleration of Na,K-ATPase) and an increase in concentrations of osmotically active metabolites (Fig. 2b). As a result, the erythrocyte volume almost does not change (Fig. 2b). If a decrease in the activity of HK is significant, the concentrations of G6P and 2,3-DPG also decrease (Fig. 2a). This is consistent with the data reported for erythrocytes of patients with hemolytic anemia associated with hexokinase deficiency [14,15,19,20,30]. The model predicts that the critical value of HK activity at which the erythrocyte loses viability is 39% of the normal activity of this enzyme. If the HK activity falls below this critical level, the cell loses the stationary state: ATP and other metabolites tend to zero, the transmembrane Na and K gradients disappear, whereas the volume increases to the critical values resulting in cell destruction (Fig. 3). The cell volume increases, however, rather slowly; therefore, erythrocytes with HK activity decreased below the critical value can remain in the circulation for several days and the ATP concentration in the erythrocyte averaged over its lifespan (what is actually measured in experiments) appears to be significantly greater than zero. The theoretical dependence of the mean ATP concentration on the HK activity in such erythrocytes (HK activity is close to or less than the critical value) is shown by the dashed line in Fig. 4.

Erythrocytes of patients with hemolytic anemia associated with HK deficiency greatly vary in intracellular ATP concentration: both greater than normal and smaller than normal values were reported (Fig. 4a) [14,15,19,20,30–35]. However, the measured values cannot be directly compared with the model results: high reticulocytosis usual in hemolytic anemia leads to overestimation of ATP concentrations and HK activities in erythrocytes. We tried to exclude the contribution of reticulocytes by assuming that it is proportional to the relative reticulocyte content in the red cell population:

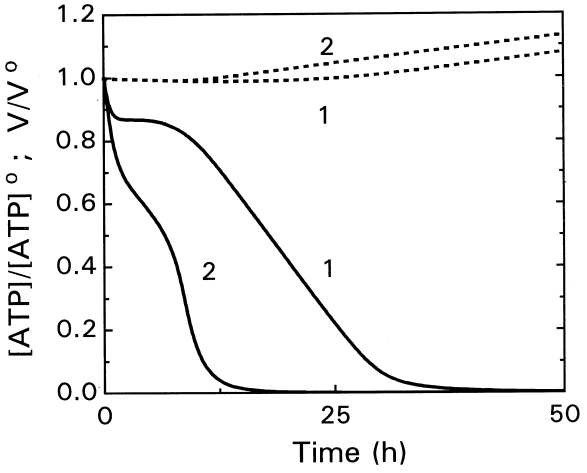

Fig. 3. Kinetics of changes in (solid line) the intracellular ATP concentration and (dashed line) erythrocyte volume after an abrupt decrease in the activity of HK from normal value to the level below the critical value. Calculations were performed for the final HK activities of 30 and 10% of the initial normal value (curves 1 and 2, respectively).

$$X = X^a + KX^aN \quad (1)$$

Here, X denotes the parameter (HK activity or ATP concentration) measured in the population of patient red blood cells; X^a is the actual value of this parameter in erythrocytes; N is the reticulocyte percentage in the population of red blood cells; and K is the coefficient. The values of K derived from the data available in the literature range from 0.074 to 0.205 for HK activity [14,15,20,30] and from 0.024 to 0.068 for ATP concentration [15,18,20]. When correcting the HK activity and ATP values reported in each of the cited studies, we used the K values derived from this particular study. The other data were corrected using the averaged K values, which were 0.143 for HK activity, and 0.043 for ATP concentration. As is evident from Fig. 4b, most of the corrected data points are close to or less than the critical value of HK activity. Our model predicts that erythrocytes lose the steady-state energy metabolism in this range of HK activities. The measurable ATP level is the mean over the erythrocyte lifespan in the circulation, rather than the steady-state concentration. The simulated (dashed line) and experimental data agree well, suggesting that inviable mature erythrocytes are in fact the cause of hemolytic anemia. This is low HK activity in these cells that makes them inviable.

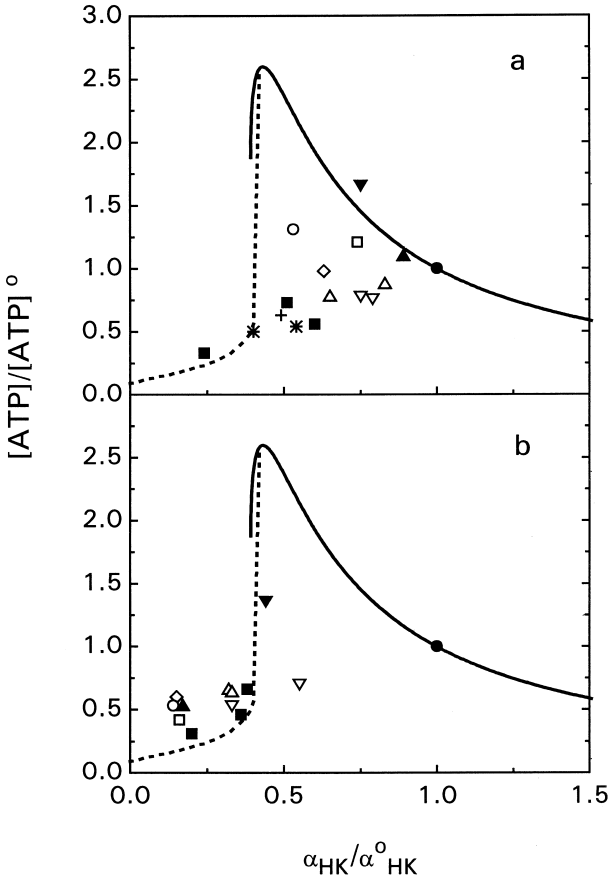

Fig. 4. ATP in erythrocytes for various levels of HK activity. The solid line indicates the calculated stable steady states. The dashed line indicates the ATP concentrations averaged over the erythrocyte lifespan (the data are calculated under the assumption that HK activity in the erythrocyte drops at zero time from the initial normal level to the level indicated in the x-axis). The erythrocyte lifespan was assumed to be either 3000 h or the time it takes the erythrocyte to increase in volume by 10%. The two curves (for the steady-state and the mean ATP concentrations) coincide at HK activities above 42% of the normal physiological level. The filled circle is the point corresponding to the normal physiological state. Symbols indicate the experimental data obtained with erythrocytes of patients with hemolytic anemia associated with HK deficiency. (a) The experimental results from: open up triangles, [14]; open circle, [15]; open square, [19]; black up triangle, [20]; open down triangles, [30]; black squares, [31]; diamond, [32]; cross, [33]; stars, [34]; black down triangle, [35]. (b) The same experimental results after subtracting the contribution of reticulocytes, estimated using the data from [14,15,18,20,32]. The results from [33,34] were not corrected because of the absence of the exact data on reticulocytosis.

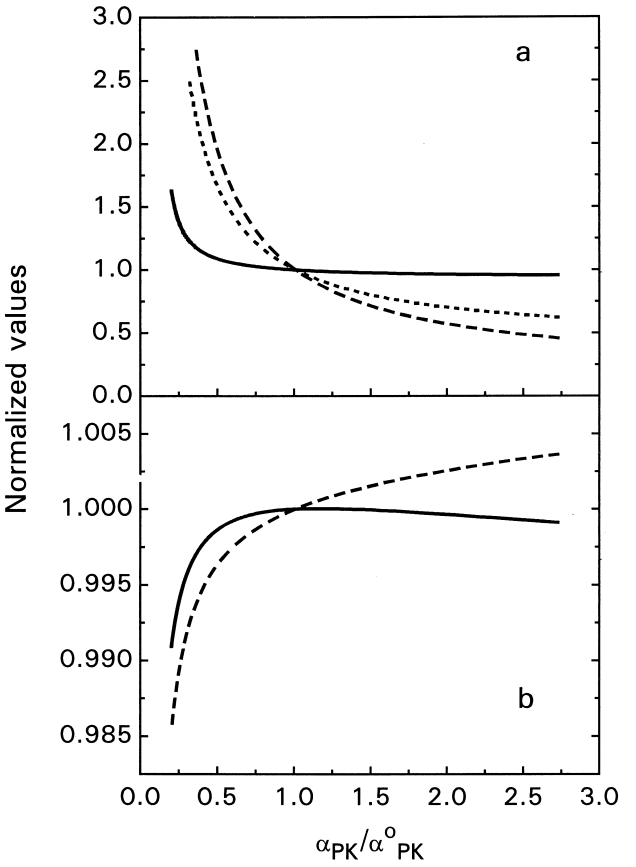

Fig. 5. Stationary normalized values of the model variables as a function of PK activity. (a) Solid line, erythrocyte volume; dashed line, 2,3-DPG concentration; and dotted line, total concentration of osmotically active metabolites. (b) Solid line, intracellular ATP and (dashed line) Na.

The results similar to those shown in Fig. 2 were obtained by varying the activities of GPI and PFK. The qualitatively different results were obtained only for G6P whose stationary concentration increased significantly with decreasing activities of these enzymes.

The results of simulation showing how PK activity affects the stationary intracellular metabolites concentrations, Na ion concentration, and erythrocyte volume are

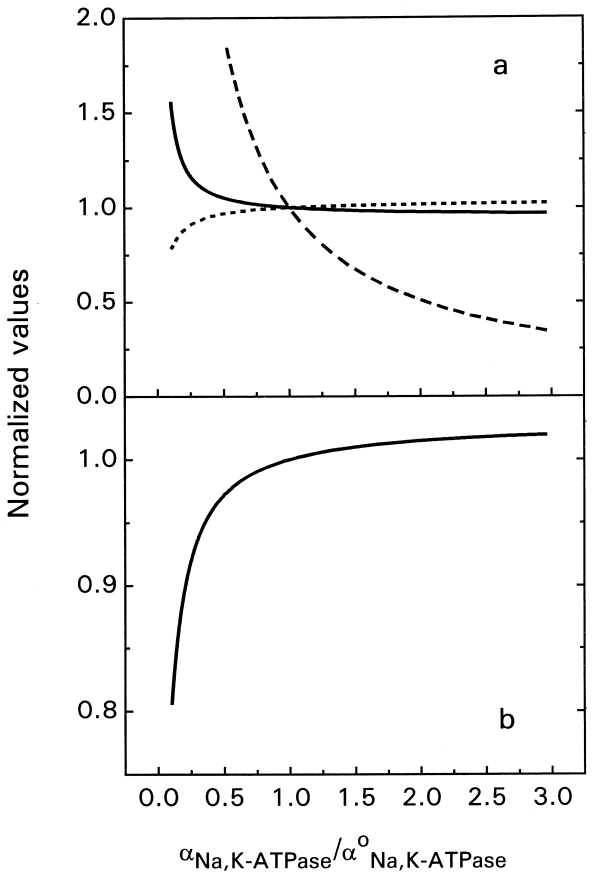

Fig. 6. Dependence of the stationary values of the model variables on the Na,K-ATPase activity. (a) solid line, erythrocyte volume; dashed line, intracellular Na; and dotted line, total concentration of osmotically active metabolites. (b) Intracellular ATP.

presented in Fig. 5. Evidently, the 10 and 50% increases in cell volume are reached at different values of PK activity. The situation observed with a deficiency of either TPI, GAPDH, PGK, DPGP, PGM, ENO or LDH was the same. If TPI, GAPDH, PGK, PGM, ENO or PK is deficient, the model predicts a slight decrease in the intracellular ATP concentration and accumulation of metabolites preceding the reaction catalyzed by the deficient enzyme, including a significant increase in DAP in TPI deficiency and an increase in 2,3-DPG in PGK, PGM, ENO or PK deficiency. These model results are qualitatively similar to the available experimental data [1–4]. Numerical analysis showed that the erythrocyte volume increases slowly in ALD, TPI, GAPDH, PGK, DPGP, PGM, ENO, PK or LDH deficiency. Therefore, the erythrocytes in which the activity of one of these enzymes is below the critical level can continue to circulate for a fairly long time (Table 2).

A decrease in the activity of transport Na,K-ATPase leads to a decrease in the stationary values of Na and K transmembrane gradients and, correspondingly, to the increase in cell volume (Fig. 6). However, if the activity of transport Na,K-ATPase increases, even greatly, the stationary Na and K transmembrane gradients increase only slightly, leaving the erythrocyte volume and metabolism almost unchanged (Fig. 6). This result is in good accordance with the experimental data obtained with erythrocytes in which Na,K-ATPase activity several times exceeds the normal value [36]. Conversely, a 3-fold increase in HK activity in the model causes the erythrocyte to increase in volume by about 10% (this means cell death; Fig. 2). The model predicts that a considerable increase in PFK, DPGM, or DPGP activity also results in an increase in erythrocyte volume and its death.

Table 1
Stationary values of intracellular concentrations and fluxes of metabolites and ions calculated for the normal physiological values of the model parameters

Variable	Calculated values	Experimental values	Units	References
[G6P]	73	20–110	μmol/l cells	[12–20]
[F6P]	24	6–15.7	μmol/l cells	[13–19]
[FDP]	7.4	2–30	μmol/l cells	[13–19,21]
[DAP]	31	7.6–35	μmol/l cells	[14,15,17,18,21,22]
[GAP]	14	4.8–20	μmol/l cells	[15,17,19,21]
[1,3-DPG]	0.72	0.40	μmol/l cells	[17]
[2,3-DPG]	4500	4170–5700	μmol/l cells	[13–17,19–21]
[3-PG]	45	55–68.5	μmol/l cells	[13–17,19]
[2-PG]	11	5.5–12.3	μmol/l cells	[13–17,19]
[PEP]	9.7	11.6–18.2	μmol/l cells	[13–17,19]
[NAD]	48	32–92	μmol/l cells	[23]
[NADH]	2.0	1–54	μmol/l cells	[23]
[ATP]	1450	1070–1830	μmol/l cells	[16–18,20]
[ADP]	240	85–300	μmol/l cells	[16–18,20]
[AMP]	39	10–50	μmol/l cells	[16,18,20]
$[Na^+]$	10	16.6 ± 1.6	mmol/l cell water	[24]
$[K^+]$	130	135 ± 5	mmol/l cell water	[24]
Glucose consumption	1.1	0.60–1.46	mmol/l cells/h	[12,14]
Lactate production	2.2	1.32–2.68	mmol/l cells/h	[12,14,25]
Na^+ efflux	2.0	2.0 ± 0.23	mmol/l cells/h	[25]
K^+ influx	1.3	1.31 ± 0.25	mmol/l cells/h	[25]

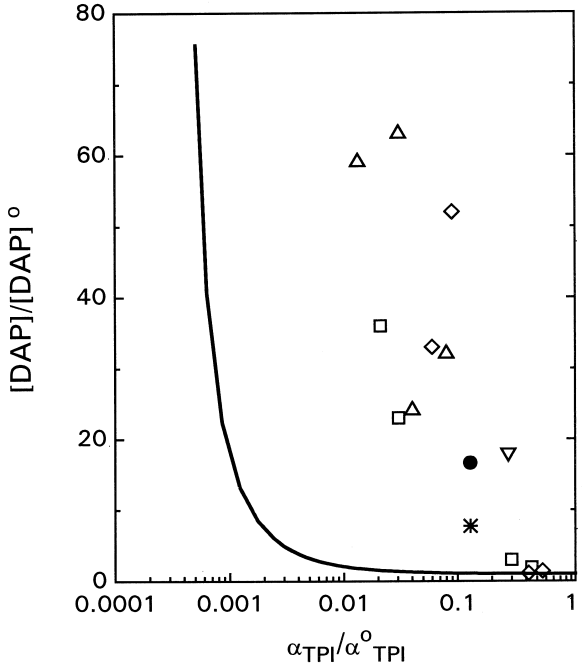

Fig. 7. DAP concentrations in erythrocytes for various levels of TPI activity. Solid line indicates the steady states calculated for the stable deficient enzyme. Star and black circle indicate the cell population means for DAP concentrations calculated for the unstable enzyme whose activity exponentially decreases with time for critical cell volumes $V/V^0 = 1.1$ and $V/V^0 = 1.5$, respectively. The calculations were made under the assumption that the concentrations of metabolites change quasi-stationarily with decreasing enzyme activity. Open symbols indicate the experimental data reported in: up triangles, [39]; down triangle, [21]; squares, [22]; and diamonds, [40].

Table 3 compares the calculated critical values of Na,K-ATPase and glycolytic enzyme activities at which cells lose viability and the experimental data on activities of deficient enzymes of patients with hemolytic anemia. The calculations were performed for stable and unstable enzymes. The stable enzymes were assumed to have low activity that is constant in time. Note that, in hemolytic anemia with HK or PK deficiency, the enzymatic activities measured in patients' erythrocytes appeared close to the calculated critical values. In contrast, the enzymatic activities measured in erythrocytes of patients with hemolytic anemia associated with GPI, PFK, TPI, or PGK deficiency were usually much greater than their calculated critical values. Hence, these enzyme deficiencies are unlikely to be the cause of erythrocyte destruction and development of anemia if the deficient enzyme is stable. Other glycolytic enzyme deficiencies are rare and no reliable evidence exists that they can be associated with hemolytic anemia.

Table 2
Time it takes erythrocytes to swell to the critical volume values after the PK activity has dropped abruptly from the normal value to the value indicated

$\alpha_{PK}/\alpha^0_{PK}$		0.4	0.2	0.1	0.05
Time (h)	$V = 1.1\ V^0$	329	54	26	19
	$V = 1.5\ V^0$	–	858	133	112

Changes in the kinetic properties of the deficient enzyme (which is characterized by only a small decrease in its activity) may account for the observed erythrocyte destruction. For instance, in some cases of PFK deficiency, ATP inhibited the deficient PFK to a greater extent than the normal enzyme [37,38]. Therefore, the rate of this enzyme in the cell appears much lower than it could be expected from the data on its activity measured by the standard method.

Earlier, we showed that the instability of a deficient enzyme may explain why, in anemia, its measured activity appears significantly greater than the calculated critical value [5]. Let us assume that the activity of the defective

Table 3
Comparison of Na,K-ATPase and glycolytic enzyme activities for which the model predicts the erythrocyte destruction and those measured in erythrocytes of patients with hemolytic anemia: Normalized critical values for stable enzyme activities (α^c/α^0;) and the mean values for unstable enzymes (α^m/α^0), calculated for two values of critical cell volume.

Enzyme	Model results				Experimental data [1–4]
	$V/V^0 = 1.1$		$V/V^0 = 1.5$		
	Stable enzyme (α^c/α^0)	Unstable enzyme (α^m/α^0)	Stable enzyme (α^c/α^0)	Unstable enzyme (α^m/α^0)	($\alpha/\alpha;^0$)
HK	0.39	0.65	0.39	0.65	0.24–0.89
GPI	0.015	0.23	0.015	0.23	0.05–0.25
PFK	0.011	0.22	0.011	0.22	0.08–0.60
ALD	0.03	0.28	0.03	0.28	0.04–0.16
TPI	0.00046	0.13	0.00039	0.13	0.016–0.30
GAPDH	0.14	0.44	0.13	0.43	0.20–0.50
PGK	0.0069	0.20	0.0033	0.17	0.01–0.30
DPGP	0.32	0.60	0.11	0.40	–
PGM	0.022	0.26	0.0074	0.20	–
ENO	0.47	0.70	0.20	0.50	0.06–0.50
PK	0.48	0.71	0.22	0.52	0.05–0.40
LDH	0.016	0.24	0.015	0.23	–
Na,K-ATPase	0.36	0.63	0.11	0.40	0.20–0.60

enzyme is normal in erythrocytes that enter the circulation, but then decays exponentially with time from the initial value (α^0) to the critical minimum (α^c):

$$\alpha = \alpha^0 \exp(-t/\tau) \quad (2)$$

Here, α is the activity of the deficient enzyme at time t, and τ is the time constant for inactivation of the enzyme. When the enzymatic activity reaches the critical minimum, an erythrocyte dies. If this is the case, the measured activity is the cell population mean, which can be significantly greater than the critical minimum. As is shown in Appendix B, under the assumptions made, the mean value (α^m) can be written as:

$$\alpha^m = (\alpha^0 - \alpha^c)/\ln(\alpha^0/\alpha^c) \quad (3)$$

As is evident from Table 3, the means calculated according to this expression are similar in most cases to the values determined experimentally in erythrocytes of patients with hemolytic anemia. Hence, the PFK and nonkey enzyme deficiencies can result in destruction of erythrocytes and development of anemia if these enzymes are unstable. Actually, the instability of the deficient enzyme can be found in most cases of hemolytic anemia associated with glycolytic enzyme deficiencies [1–4]. The suggestion that the activity of a defective enzyme decreases exponentially is not critical. This decrease can follow an arbitrary law. The suggestion that the deficient enzyme is unstable also makes it possible to describe quantitatively certain changes in metabolite concentrations observed in patient erythrocytes (e.g. extremely high DAP concentration in TPI deficiency). The DAP concentration calculated according to the model for the values of TPI activities observed in patient erythrocytes does not differ from the DAP concentration in normal erythrocytes if the deficient enzyme is assumed to be stable. In fact, patient erythrocytes contain DAP at concentrations exceeding the normal one by 20–60 times [21,22,39,40]. For unstable TPI, the calculated DAP concentration averaged over cell population agrees well with the values measured in patient erythrocytes (Fig. 7).

It is of interest that the calculated enzymatic activities that correspond to the increases in the erythrocyte volume by 10 and 50% are not very different (Table 3). In fact, they coincide to an order of magnitude accuracy. Hence, the exact value of the critical volume is not crucial for determining the critical values of enzymatic activities. Moreover, the critical values of glycolytic enzyme activities obtained in [5,6] and in this study are similar to a great extent, despite that the criteria used for the viability of erythrocytes were different. The calculated values of ATPase and glycolytic enzyme activities at which cells lose the viability are in a good agreement with the experimental data on activities of deficient enzymes of patients with hemolytic anemia. This suggests that the existing mathematical models can be a successful tool for analysis of particular cases of hemolytic anemia caused by glycolytic enzyme deficiencies. It is important for this analysis that such factors as changes in the kinetic parameters of the deficient enzyme, its instability, and the distribution of the experimentally determined parameters in the erythrocytes population were taken into account.

Acknowledgements

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Appendix A. Mathematical model

A.1. Energy metabolism

Energy metabolism involves ATP production and consumption in various energy-dependent processes. In erythrocytes, ATP is generated in anaerobic glycolysis (Fig. 1) and consumed in various ATP-utilizing reactions. The glycolysis rate is under control of ATP consumption; this makes it possible to maintain the high level of cell energy charge in a wide range of ATP consumption rate [12].

A.1.1. Glycolysis

In the model, glycolysis is described by the following set of equations:

$$\frac{d}{dt}\left([G6P]\frac{V}{V^0}\right) = v_{HK} - v_{GPI} \quad (4)$$

$$\frac{d}{dt}\left([F6P]\frac{V}{V^0}\right) = v_{GPI} - v_{PFK} \quad (5)$$

$$\frac{d}{dt}\left([FDP]\frac{V}{V^0}\right) = v_{PFK} - v_{ALD} \quad (6)$$

$$\frac{d}{dt}\left([DAP]\frac{V}{V^0}\right) = v_{ALD} - v_{TPI} \quad (7)$$

$$\frac{d}{dt}\left([GAP]\frac{V}{V^0}\right) = v_{ALD} + v_{TPI} - v_{GAPDH} \quad (8)$$

$$\frac{d}{dt}\left([1,3-DPG]\frac{V}{V^0}\right) = v_{GAPDH} - v_{PGK} - v_{DPGM} \quad (9)$$

$$\frac{d}{dt}\left([2,3-DPG]\frac{V}{V^0}\right) = v_{DPGM} - v_{DPGP} \quad (10)$$

$$\frac{d}{dt}\left([3-PG]\frac{V}{V^0}\right) = v_{PGK} - v_{PGM} + v_{DPGP} \quad (11)$$

$$\frac{d}{dt}\left([2-PG]\frac{V}{V^0}\right) = v_{PGM} - v_{ENO} \quad (12)$$

$$\frac{d}{dt}\left([PEP]\frac{V}{V^0}\right) = \upsilon_{ENO} - \upsilon_{PK} \tag{13}$$

$$\frac{d}{dt}\left([NADH]\frac{V}{V^0}\right) = \upsilon_{GAPDH} - \upsilon_{LDH} \tag{14}$$

where V and V^0 are the current and the normal physiological cell volumes (here and hereafter, the physiological (normal) values of the parameters and variables are marked by '0'). The υ_{HK}, υ_{GPI}, υ_{PFK}, υ_{ALD}, υ_{TPI}, υ_{GAPDH}, υ_{PGK}, υ_{DPGM}, υ_{DPGP}, υ_{PGM}, υ_{ENO}, υ_{PK}, υ_{LDH} are the rates of the corresponding enzymatic reactions.

In the model, glycolysis is independent of the glucose concentration, because normally, in vivo, erythrocyte glycolysis is always saturated with glucose. The concentrations of orthophosphate, pyruvate, lactate, and the total amount of NAD and NADH in the model are also constant and equal to 1, 0.07, 1.2, and 0.05 mM, respectively [13–17,23].

The expressions for the enzyme reaction rates and the parameter values were taken from [5,41–43]:

$$\upsilon_{HK} = \alpha_{HK}\frac{[ATP]/K^1_{HK}}{1 + [ATP]/K^1_{HK} + [G6P]/K^2_{HK}} \tag{15}$$

$\alpha^0_{HK} = 12$ mM/h, $K^1_{HK} = 1$ mM, $K^2_{HK} = 5.5\cdot10^{-3}$ mM,

$$\upsilon_{GPI} = \alpha_{GPI}\frac{([G6P] - [F6P]K^1_{GPI})/K^2_{GPI}}{1 + [G6P]/K^2_{GPI} + [F6P]/K^3_{GPI}} \tag{16}$$

$\alpha^0_{GPI} = 360$ mM/h, $K^1_{GPI} = 3$, $K^2_{GPI} = 0.3$ mM, $K^3_{GPI} = 0.2$ mM.

$$\upsilon_{PFK} = \alpha_{PFK}\frac{1.1\cdot[ATP][F6P]}{(K^2_{PFK} + [ATP])(K^1_{PFK} + [F6P])}\frac{[1/(1 + [AMP]/K^3_{PFK}) + 2[AMP]/(K^3_{PFK} + [AMP])]}{\left[1 + 10^8\dfrac{(1 + [ATP]/K^4_{PFK})^4}{(1 + [AMP]/K^3_{PFK})^4(1 + [F6P]/K^5_{PFK})^4}\right]} \tag{17}$$

$\alpha^0_{PFK} = 380$ mM/h, $K^1_{PFK} = 0.1$ mM, $K^2_{PFK} = 2$ mM, $K^3_{PFK} = 10^{-2}$ mM, $K^4_{PFK} = 19.5\cdot10^{-2}$ mM, $K^5_{PFK} = 3.7\cdot10^{-4}$ mM.

$$\upsilon_{ALD} = \alpha_{ALD}\frac{[FDP]/K^1_{ALD} - [DAP][GAP]/K^2_{ALD}}{1 + \dfrac{[FDP]}{K^3_{ALD}} + \dfrac{[DAP]}{K^4_{ALD}} + \dfrac{[GAP]}{K^5_{ALD}} + \dfrac{[FDP][DAP]}{K^3_{ALD}K^4_{ALD}} + \dfrac{[DAP]^2}{K^4_{ALD}K^6_{ALD}} + \dfrac{[DAP][GAP]}{K^4_{ALD}K^7_{ALD}}} \tag{18}$$

$\alpha^0_{ALD} = 76$ mM/h, $K^1_{ALD} = 10^{-2}$ mM, $K^2_{ALD} = 6\cdot10^{-4}$ mM^2, $K^3_{ALD} = 10^{-2}$ mM, $K^4_{ALD} = 3.2\cdot10^{-2}$ mM, $K^5_{ALD} = 2.1\cdot10^{-3}$ mM, $K^6_{ALD} = 2$ mM, $K^7_{ALD} = 6.5\cdot10^{-2}$ mM.

$$\upsilon_{TPI} = \alpha_{TPI}\frac{([DAP] - [GAP]/K^2_{TPI})/K^1_{TPI}}{1 + [DAP]/K^1_{TPI} + [GAP]/K^3_{TPI}} \tag{19}$$

$\alpha^0_{TPI} = 3000$ mM/h, $K^1_{TPI} = 0.82$ mM, $K^2_{TPI} = 0.45$ mM, $K^3_{TPI} = 0.43$ mM.

$$\upsilon_{GAPDH} = \alpha_{GAPDH}\frac{([GAP][NAD][P_i] - [1,3-DPG][NADH]/K^4_{GAPDH})/K^1_{GAPDH}K^2_{GAPDH}K^3_{GAPDH}}{1.29\left(1 + \dfrac{[GAP]}{K^1_{GAPDH}} + \dfrac{[1,3-DPG]}{K^5_{GAPDH}}\right)\left(1 + \dfrac{[NAD]}{K^2_{GAPDH}} + \dfrac{[NADH]}{K^6_{GAPDH}}\right)} \tag{20}$$

$\alpha^0_{GAPDH} = 690$ mM/h, $K^1_{GAPDH} = 0.13$ mM, $K^2_{GAPDH} = 0.13$ mM, $K^3_{GAPDH} = 3.4$ mM, $K^4_{GAPDH} = 0.136$ mM^{-1}, $K^5_{GAPDH} = 1.3\cdot10^{-2}$ mM, $K^6_{GAPDH} = 2\cdot10^{-3}$ mM.

$$\upsilon_{PGK} = \alpha_{PGK}\frac{([1,3-DPG][ATP] - [3-PG][ADP]/K^3_{PGK})/K^1_{PGK}K^2_{PGK}}{(1 + [ATP]/K^5_{PGK} + [ADP]/K^2_{PGK} + A[1,3-DPG]/K^1_{PGK} + B[3-PG]/K^6_{PGK})} \tag{21}$$

$A = (K^4_{PGK} + [ADP] + K^4_{PGK}[ATP]/K^5_{PGK})/K^2_{PGK}$; $B = (K^7_{PGK} + [ATP] + K^7_{PGK}[ADP]/K^2_{PGK})/K^5_{PGK}$; $\alpha^0_{PGK} = 7330$ mM/h, $K^1_{PGK} = 2.2\cdot10^{-3}$ mM, $K^2_{PGK} = 0.14$ mM, $K^3_{PGK} = 380$ mM, $K^4_{PGK} = 0.3$ mM, $K^5_{PGK} = 0.27$ mM, $K^6_{PGK} = 1.4$ mM, $K^7_{PGK} = 0.4$ mM.

$$\upsilon_{DPGM} = \alpha_{DPGM}\frac{[1,3-DPG]}{K^1_{DPGM} + K^2_{DPGM}[1,3-DPG] + [2,3-DPG]} \quad (22)$$

$\alpha^0_{DPGM} = 3892$ mM/h, $K^1_{DPGM} = 0.04$ mM, $K^2_{DPGM} = 1.3{\cdot}10^{-2}$.

$$\upsilon_{DPGP} = \alpha_{DPGP}\frac{[2,3-DPG]}{[2,3-DPG] + K^1_{DPGP}(1 + ([2-PG] + [3-PG])/K^2_{DPGP})} \quad (23)$$

$\alpha^0_{DPGP} = 0.65$ mM/h, $K^1_{DPGP} = 0.02$ mM, $K^2_{DPGP} = 6{\cdot}10^{-3}$ mM.

$$\upsilon_{PGM} = \alpha_{PGM}\frac{([3-PG] - [2-PG]/K^2_{PGM})/K^1_{PGM}}{1 + [3-PG]/K^1_{PGM} + [2-PG]/K^3_{PGM}} \quad (24)$$

$\alpha^0_{PGM} = 1100$ mM/h, $K^1_{PGM} = 0.27$ mM, $K^2_{PGM} = 0.24$ mM, $K^3_{PGM} = 0.02$ mM.

$$\upsilon_{ENO} = \alpha_{ENO}\frac{([2-PG] - [PEP]/K^2_{ENO})/K^1_{ENO}}{1 + [2-PG]/K^1_{ENO} + [PEP]/K^3_{ENO}} \quad (25)$$

$\alpha^0_{ENO} = 83$ mM/h, $K^1_{ENO} = 0.056$ mM, $K^2_{ENO} = 6.7$, $K^3_{ENO} = 2{\cdot}10^{-3}$ mM.

$$\upsilon_{PK} = \alpha_{PK}\frac{[PEP][ADP]/K^1_{PK}K^2_{PK}}{1 + [ATP]/K^3_{PK} + [ADP]/K^2_{PK} + [PEP]/K^1_{PK} + [PEP][ADP]/(K^1_{PK}K^2_{PK})} \quad (26)$$

$\alpha^0_{PK} = 120$ mM/h, $K^1_{PK} = 0.05$ mM, $K^2_{PK} = 0.42$ mM, $K^3_{PK} = 0.35$ mM.

$$\upsilon_{LDH} = \alpha_{LDH}\frac{([PYR][NADH] - [LAC][NAD]/K^3_{LDH})/K^1_{LDH}K^2_{LDH}}{1 + \frac{[PYR]}{K^1_{LDH}}\frac{[NADH]K^4_{LDH} + [PYR][NADH] + [LAC][NADH]K^4_{LDH}/K^5_{LDH}}{K^1_{LDH}K^2_{LDH}} + \frac{A}{K^5_{LDH}K^6_{LDH}}} \quad (27)$$

$A = [NAD]K^7_{LDH} + [LAC]K^6_{LDH} + [NAD][LAC] + [PYR][NAD]K^7_{LDH}/K^1_{LDH}$; $\alpha^0_{LDH} = 550$ mM/h, $K^1_{LDH} = 0.022$ mM, $K^2_{LDH} = 0.007$ mM, $K^3_{LDH} = 426$, $K^4_{LDH} = 0.14$ mM, $K^5_{LDH} = 380$ mM, $K^6_{LDH} = 0.1$ mM, $K^7_{LDH} = 170$ mM.

A.1.2. ATPases

An appreciable fraction of ATP produced in erythrocytes is consumed by Na,K-ATPase for which the reaction rate was written in the model as:

$$\upsilon_{Na,K-ATPase} = \alpha_{Na,K-ATPase}[Na^+][ATP] \quad (28)$$

$\alpha^0_{Na,K-ATPase} = 0.045$ l/(h·mM)

The ATP consumption by Na,K-ATPase was experimentally estimated at about 50% of the glycolytically produced ATP [12,25,44]. The remaining ATP is expended for processes other than transmembrane Na^+ and K^+ transport. It is of interest that the major ATP consumers have not yet been identified in erythrocytes. As in other models [43,45,46], the ATP utilization in processes other than ion transport is described in our model by introducing an additional ATPase.

$$\upsilon_{ATPase} = \alpha_{ATPase}[ATP]/([ATP] + K_{ATPase}) \quad (29)$$

$\alpha_{ATPase} = 1.6$ mM/h, $K_{ATPase} = 1$ mM.

A.2. Adenylate metabolism

Adenylate metabolism determines the adenylate pool value and, thereby, the absolute adenine nucleotide concentrations in erythrocytes. Changes in the erythrocyte adenylate pool value result from AMP synthesis and degradation [47–49]. These processes are relatively slow in erythrocytes [50–54]; however, in long-term pathologies, they may lead to considerable changes in the adenylate pool and adenine nucleotide concentrations [55–60].

Therefore, we included the terms describing adenylate metabolism into the model: a reaction of irreversible AMP synthesis and two irreversible reactions of AMP degradation. The dynamics of adenylate nucleotide concentrations is determined by the following set of equations describing the interaction of adenylate and energy metabolisms:

$$\frac{d}{dt}\left([ATP]\frac{V}{V^0}\right) = \upsilon_{PGK} + \upsilon_{PK} - \upsilon_{HK} - \upsilon_{Na,K-ATPase} - \upsilon_{ATPase} - \frac{3}{2}\upsilon_s - \upsilon_{AK} \quad (30)$$

$$\frac{d}{dt}\left([ADP]\frac{V}{V^0}\right) = -\upsilon_{PGK} - \upsilon_{PK} + \upsilon_{PFK} + \upsilon_{HK} + \upsilon_{Na,K-ATPase} + \upsilon_{ATPase} + \upsilon_s + 2\upsilon_{AK} \quad (31)$$

$$\frac{d}{dt}\left([AMP]\frac{V}{V^0}\right) = \frac{3}{2}\upsilon_s - \upsilon_{AMPP} - \upsilon_{AMPD} - \upsilon_{AK} \quad (32)$$

$$\frac{[ADP]^2}{[AMP][ATP]} = 1 \quad (33)$$

Here, υ_s is the rate of AMP production in adenosine kinase and adenine phosphoribosyl transferase reactions. We assume that the rates of these two reactions are equal and constant. Eqs. 30–32 are written according to the stoichiometry of adenylate formation and consumption during AMP synthesis from adenine and adenosine; υ_{AK} is the rate of adenylate kinase reaction υ_{AMPP} and υ_{AMPD} denote the rates of AMP phosphatase (AMPP) and AMP deaminase (AMPD) reactions. Note that υ_s is assumed to be constant (υ_s = constant = 0.04 mM/h). This low rate of adenylate metabolism in human erythrocytes (about 4% of glycolytic flux) is optimum for maintaining the stable state of cellular metabolism [28].

The rate of AMP degradation in AMPD reaction is taken in the form:

$$\upsilon_{AMPD} = \alpha_{AMPD}\left(\frac{[AMP]}{[AMP] + K^1_{AMPD}}\right)^4, \quad (34)$$

where α^0_{AMPD} = 48 mM/h, and K^1_{AMPD} = 1 mM [61–63].

This is the sigmoid dependence of the reaction rate on the concentration of AMP known from the literature [62–64]. The sigmoid kinetics of AMPD implies that it almost does not operate at normal intracellular concentrations of AMP. We suggest that under physiological conditions, AMP degradation is regulated by AMPP. The rate of AMPP reaction is taken as:

$$\upsilon_{AMPP} = \alpha_{AMPP}\frac{1 + [ATP]/K^2_{AMPP}}{(1 + K^1_{AMPP}/[AMP] + [AMP]/K^3_{AMPP})} \quad (35)$$

α^0_{AMPP} = 0.011 mM/h, $K^1_{AMPP} = 10^{-5}$ mM, K^2_{AMPP} = 0.01 mM, K^3_{AMPP} = 0.0001 mM.

As is evident from this expression, the AMPP reaction rate is directly proportional to the ATP concentration and inversely proportional to the AMP concentration in a broad range of AMP and ATP concentrations. By using mathematical modeling, we demonstrate that this form of the dependence provides the most efficient stabilization of the intracellular ion concentrations and the cell volume when the parameters of the cell vary [28,29]. The α_{AMPP} value was chosen to ensure the normal stationary concentrations of adenine nucleotides in the model. The expression for the AMPP reaction rate is hypothetical; it was obtained by optimizing the mathematical models of the erythrocyte [28]. The kinetics of this enzyme under intracellular conditions has been studied insufficiently. However, in vitro, the rate of purified rat liver AMPP exhibited a bell-shaped dependence on the energy charge [65]. Since an increase in the energy charge implies an increase in the ATP concentration and a decrease in the AMP concentration, it is plausible that the rate of AMPP reaction is proportional to the ATP concentration and inversely proportional to the AMP concentration within some range of intracellular adenine nucleotide concentrations.

Adenylate kinase reaction is believed to be in equilibrium (Eq. 33) because of the high activity of adenylate kinase in erythrocytes [66,67]. The equilibrium in adenylate kinase reaction allows us to exclude the rate of adenylate kinase from Eqs. 30–32.

A.3. Ion transport, osmotic balance, and cell volume

The description of transmembrane ion transport is essentially the same as in [29]. The differential equations for transmembrane Na^+ and K^+ fluxes were written under the assumption that the equilibrium distribution of permeating anions exists between the cell and the medium.

$$\frac{d}{dt}\left([K^+]_i\frac{V}{V^0}\right) = 2\upsilon_{Na,K-ATPase} + J_K;$$

$$J_K = P_K\frac{\frac{\varphi F}{R\theta}}{\exp\left(\frac{\varphi F}{R\theta}\right) - 1}\left([K^+]_e - [K^+]_i\exp\left(\frac{\varphi F}{R\theta}\right)\right) \quad (36)$$

$$\frac{d}{dt}\left([Na^+]_i\frac{V}{V^0}\right) = -3\upsilon_{Na,K-ATPase} + J_{Na};\ J_{Na} =$$

$$P_{Na}\frac{\frac{\varphi F}{R\theta}}{\exp\left(\frac{\varphi F}{R\theta}\right) - 1}\left([Na^+]_e - [Na^+]_i\exp\left(\frac{\varphi F}{R\theta}\right)\right) \quad (37)$$

$$\frac{[A^-_p]_e}{[A^-_p]_i} = \exp\left(-\frac{\varphi F}{R\theta}\right) \quad (38)$$

Here, J_K and J_{Na} denote passive K^+ and Na^+ fluxes through the erythrocyte membrane; P_K and P_{Na} are the

passive permeabilities of the erythrocyte membrane to K^+ and Na^+, respectively; F is the Faraday constant; R is the universal gas constant; θ denotes absolute temperature; φ is the transmembrane potential; $[A_p^-]$ is the total concentration of permeating anions (Cl^- and HCO_3^-); and subscripts i and e indicate intra- and extracellular ion concentrations, respectively. The parameters in these equations were as follows [29]: $P_K = 1.24 \cdot 10^{-2}$ l/h; $P_{Na} = 1.22 \cdot 10^{-2}$ l/h; $[K^+]_e = 5$ mM; $[Na^+]_e = 145$ mM; $[A^-]_e = 150$ mM.

The model also included the equations for intracellular content electroneutrality and osmotic balance between the cell and the medium.

$$[K^+]_i + [Na^+]_i - [A^-]_i + ZW = 0 \tag{39}$$

$$[K^+]_i + [Na^+]_i + [A^-]_i + \Omega + W = [K^+]_e + [Na^+]_e + [A^-]_e = 2L = 300 \text{ mM} \tag{40}$$

Here, W and Z are the sum of the concentrations of hemoglobin and other osmotically active non-permeating substances in the cell (excluding glycolytic metabolites and adenylates), and their average charge, respectively; Ω denotes the sum of concentrations of non-permeating glycolytic metabolites and adenylates:

$$\Omega = [G6P] + [F6P] + [FDP] + [DAP] + [GAP] + [1,3-DPG] + [2,3-DPG] + [3-PG] + [2-PG] + [PEP] + [ATP] + [ADP] + [AMP]$$

Given the normal values of the model parameters, $W = 43.4$ mM; $Z = -0.7$.

The equation describing the kinetics of changes in the cell volume can be derived from Eqs. 36–40. Combining Eqs. 36 and 37, we obtain:

$$\frac{d}{dt}\left([K^+]\frac{V}{V^0} + [Na^+]\frac{V}{V^0}\right) = -\upsilon_{Na,K-ATPase} + J_K + J_{Na} \tag{41}$$

The sum of Eqs. 39 and 40 is:

$$[K^+] + [Na^+] = L - \frac{1}{2}W(1+Z) - \frac{1}{2}\Omega \tag{42}$$

Multiplying this equation by V/V^0 and differentiating the product with respect to time leads to:

$$\frac{d}{dt}\left([K^+]\frac{V}{V^0} + [Na^+]\frac{V}{V^0}\right) = L\frac{d}{dt}\left(\frac{V}{V^0}\right) - \frac{1}{2}(1+Z)\frac{d}{dt}\left(W\frac{V}{V^0}\right) - \frac{1}{2}\frac{d}{dt}\left(\Omega\frac{V}{V^0}\right) = L\frac{d}{dt}\left(\frac{V}{V^0}\right) - \frac{1}{2}\frac{d}{dt}\left(\Omega\frac{V}{V^0}\right) \tag{43}$$

Equating the right-hand parts of Eqs. 41 and 43 yields the resultant equation for the kinetics of cell volume:

$$\frac{d}{dt}\left(\frac{V}{V^0}\right) = \frac{-\upsilon_{Na,K-ATPase} + J_K + J_{Na} + \frac{1}{2}\frac{d}{dt}\left(\Omega\frac{V}{V^0}\right)}{L} \tag{44}$$

We used the DBSolve software [68] based on the modified Gear algorithm [69] and the parameter-continuation algorithm for numerical analysis of dynamic behavior and stationary states of the model, respectively.

Appendix B. The development of the expression for the mean value of activity of an unstable enzyme over an erythrocyte population

The erythrocyte lifespan in the circulation (T) is related to the critical value of enzymatic activity (α^c) by the following expression derived from Eq. 2:

$$\alpha^c = \alpha^0 \exp(T/\tau) \tag{45}$$

It can be rewritten in the following form:

$$\ln(\alpha^c/\alpha^0) = -T/\tau \text{ and } T = \tau\ln(\alpha^0/\alpha^c) \tag{46}$$

The activity of an unstable enzyme averaged over its lifespan then will be:

$$\alpha^m = \frac{1}{T}\int_0^T \alpha\,dt = \frac{1}{T}\int_0^T \alpha^0\exp(-t/\tau)dt = \frac{1}{\tau\ln(\alpha^0/\alpha^c)}(\tau\alpha^0\exp(-t/\tau)|_0 - \tau\alpha^0\exp(-t/\tau)|_T) = \frac{1}{\ln(\alpha^0/\alpha^c)}(\alpha^0\exp(-0/\tau) - \alpha^0\exp(-T/\tau)) = (\alpha^0 - \alpha^c)/\ln(\alpha^0/\alpha^c) \tag{47}$$

If the situation is stationary, every erythrocyte age is equally represented in the population of circulating cells (equal numbers of erythrocytes in each age cohort). In this case, the expression obtained also describes the mean activity of an unstable enzyme for an erythrocyte population.

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