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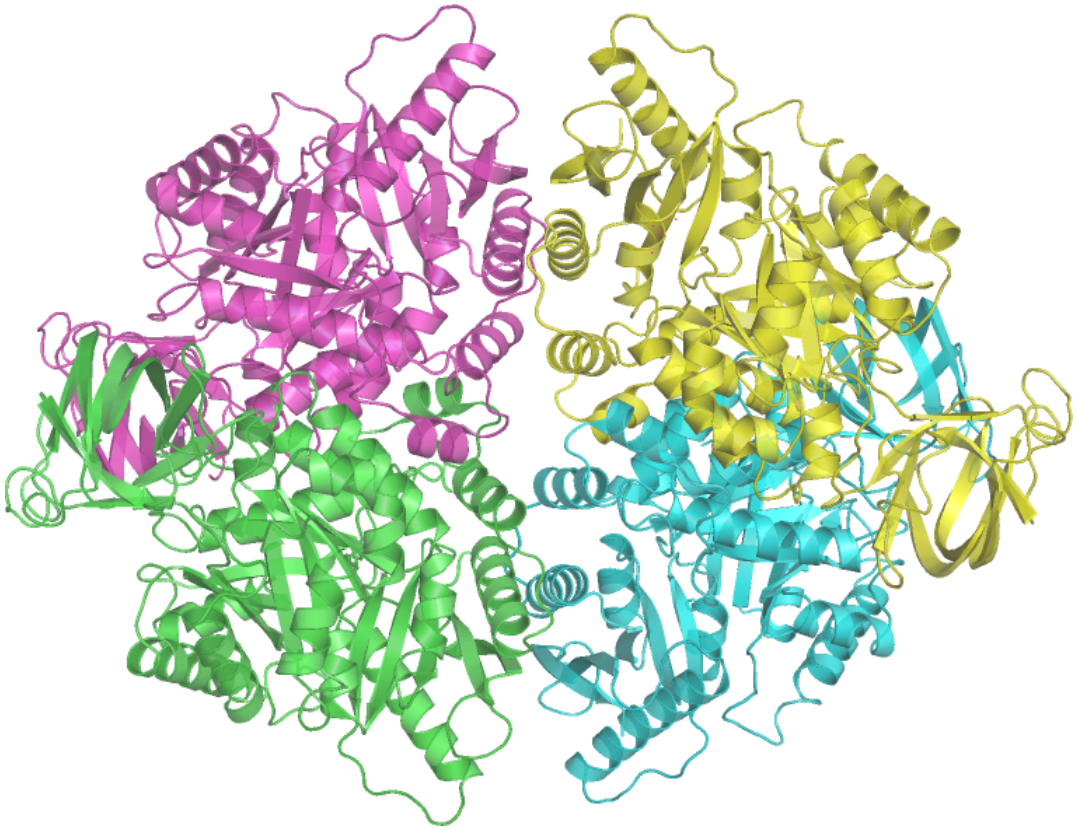


Felix To - A09982183
Orysya Stus - A10743411

Pyruvate Kinase Deficiency Simulation

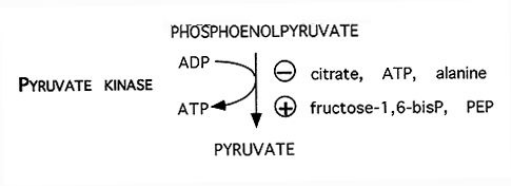
Abstract

Through the use of dynamic simulation pyruvate kinase (PK) deficiency can be simulated and analyzed using an in silico model of the red blood cell (RBC). Through the use of Mathematica and the MASS Toolbox, two models were created: the healthy RBC model showing normal metabolic processes and the diseased PK deficiency RBC model with 0.99 mM ATP consumption, 750% increase in oxygen consumption, and 17% decrease in water consumption [1]. Algorithmic analysis concluded that PK deficiency results in decreased ATP production and energy retention ability. The quantitative results correlate to the following phenotypic expression of the disease: hemolytic anemia and jaundice.

Introduction

The red blood cell (RBC) in silico model includes 36 dynamic, independent variables described by mass conservation which take into consideration the following biochemical components: glycolysis, pentose phosphate pathway, salvage pathways, and hemoglobin. Phosphofructokinase and pyruvate kinase were included in the RBC model in order to consider the effects of metabolites on the allosteric inhibition and regulation of both enzymes.

The MASS model in Mathematica includes the stoichiometric matrix, equilibrium constants, and steady-state flux and concentrations of human RBC metabolism. The model allows for the ability to perform time scale decomposition, dynamic autocorrections between metabolites or fluxes, and the use of mathematical techniques to find biological relevance in the models. After creating an in silico model, concentration and flux perturbations can be introduced in order to examine whether the ATP energy charge remains under different disturbances to mathematical ones (ie. the extent to which linearization is near the steady state is appropriate to approximate dynamic responses. The perturbations were simulated using Mathematica and the graphs and data were analyzed for physiological relevance.



Pyruvate kinase acts as the regulatory enzyme (one of the key key ones) responsible for the conversion of PEP to pyruvate in glycolysis. It is a tetramer with metal binding sites on each submit for Mg2+. It is allosterically inhibited by citrate, ATP, and alanine and activated by fructose 1,6-bisphosphate.

Pyruvate Kinase deficiency is the most common nonspherocytic hemolytic anemia among several red cell enzyme defects of the glycolytic pathway. If the RBC contains abnormally low levels of pyruvate kinase, then the production of pyruvate is limited. This causes a decreased amount of ATP production in the RBC while previous products of glycolysis increase causing an abnormal build up in the RBC. An accumulation of the glycolytic intermediates, specifically 2,3-PG, may increase up to three-fold and further impair the glycolytic flux by inhibiting hexokinase and decreasing hemoglobin oxygen affinity. Hemolytic anemia where contents of the cell are released, specifically iron and bilirubin (causes jaundice), from the cell or destruction of these abnormal red blood cells by the spleen can result. For the purpose of this project, we are considering the severe phenotypic expression of PK deficiency with hemoglobin levels lower than 8 g/dL.

The biological mechanism responsible for pyruvate kinase deficiency is located on the

PK-LR gene (over 9.5 kb) on chromosome 1q21. About 180 mutations associated with non-sperocytic haemolytic anaemia and 9 polymorphic sites have been reported in the PK-LR gene, the most common mutations include: missense (69%), splicing (13%), and stop codon (5%). The most frequent mutations for PK deficiency include: 1529A and 1456T with 721T.

Method

The MASS (Mass Action Stoichiometric Simulation) Toolbox uploaded into the software used for this project, Wolfram Mathematica, provided the metabolite concentrations and reaction rate in the RBC model. This tool allowed for the integration of desired metabolic processes and for the appropriate PK deficiency perturbations on our healthy RBC model. The following perturbations to produce the diseased PK deficiency RBC model were: 0.99 mM ATP consumption, 750% increase in oxygen consumption, and 17% decrease in water consumption

Two models were constructed: one of a healthy RBC (through the integration of the appropriate metabolic pathways) and another of a diseased RBC. The following algorithms were considered in order to asses the abnormalities in metabolism in severe cases of PK deficiency:

$$\text{charge} = \frac{\text{occupancy}}{\text{capacity}}$$

Ratios of energy charge were compared to show that PK-deficient red cells are unable to tolerate the energy load and will subsequently lyse.

$R_0 \rightleftharpoons T_0.$

$$L = \frac{T_0}{R_0}$$

The PK Relaxed ratios between our healthy and diseases model showed a comparison of the amount of PK red blood cells in the relaxed state. This ratio allows us to observe how well PK-deficient RBC binds to ADP and PEP. A large ratio would mean high PK activity and ability to catalyze the last step of glycolysis.

$$r_R = \frac{\sum_{i=0}^{4}(R_i + R_{i,A} + R_{i,AF})}{PFK_{tot}}.$$

A comparison of the Hemoglobin T/R ratios shows a ratio between the catalytically tense (inactive) and relaxed (active) states of PK in both models. (In calculations, PK not PFK was used).

Results

The physiological perturbations chosen were to increase ATP consumption by 20% and to decrease the external oxygen concentration by 75%. These perturbations were selected to model a person performing strenuous exercise. The rate constant for ATP usage was multiplied by a factor of 1.2 in the healthy model. The oxygen concentration in the healthy model was also multiplied by a factor 0.25 to simulate such conditions. These same deviations were applied to the diseased model. The data reveals that a healthy RBC displays an increase in PK activity to generate more ATP to as a response, whereas a diseased RBC displays lower levels of PK activity. This is observed by the difference in initial and final ratios in **Figure 1**.

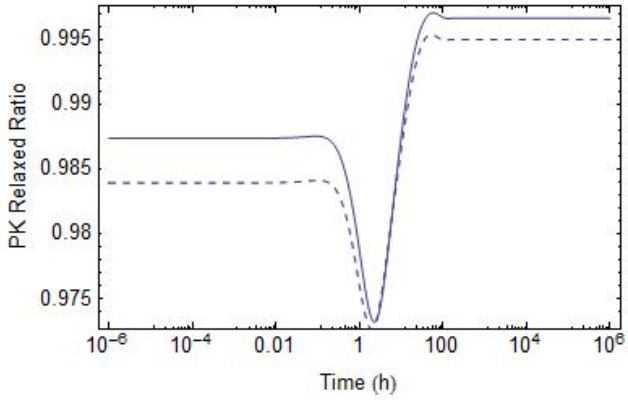


Figure 1 comparison - the pyruvate kinase relaxed ratio of the healthy (solid) and diseased (dashed) models. The diseased model is shown to display lower PK activity.

A diseased RBC, plotted alongside the healthy RBC, also displays lower energy charge levels during exercise due to a decrease in ability to retain its charged phosphates. This is displayed in **Figure 2**. The dashed line has a lower initial and final energy charge ratio than the solid line.

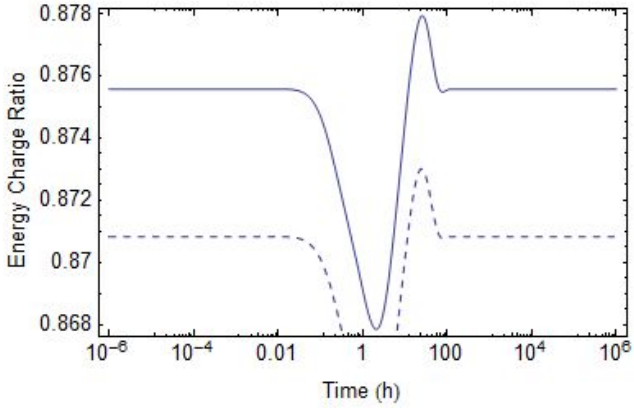


Figure 2 - The energy loading capacity of a healthy (solid) and diseased (dashed) PK model.

Discussion

In the first perturbation, the ATP consumption rate was increased by 20%. An increase of ATP usage occurs during physical activity as the body is trying to produce ATP to satisfy increased muscle activity. PK catalyzes the last step of glycolysis and enables the body to ultimately make more ATP. In Figure 1, it can be seen that the dashed line (diseased model) has lower initial and final values than the solid line (healthy model). From this data, we deduce that there are more bound R sites in a healthy RBC than a diseased RBC. This is significant because it shows that PK-deficient RBCs have fewer catalytic states. PK-deficient RBCs cannot catalyze as rapidly as healthy RBCs which leads to an overall decreased generation of necessary ATP.

For the second perturbation, the external oxygen concentration was decreased by 75% to mimic the rapid usage of oxygen in cellular respiration. The ATP energy charge (EC) ratio between the healthy and diseased models is shown in Figure 2. The EC ratio allows one to observe how many phosphates are occupied by ATPs. This is significant because ATP retention ability can be observed. It can be seen that the diseased model has a decreased ability (lower EC

ratio) to hold ATP. It has been shown that cells deficient in ATP can rapidly undergo hemolysis.

The diseased model constructed shows that a person with PK deficiency has decreased ATP production and ability to handle the energy load. Cells deficient in ATP will deform and lead to a build-up of reaction intermediates. This increases the levels of 2,3 BPG and further decrease the oxygen affinity. Our results show that people with PK deficiency have an increased risk of hemolytic anemia and jaundice. Due to such issues, PK-deficient patients must often receive blood transfusions or spleen surgery depending on the severity of the disease according to clinical data. Our simulations show that a PK-deficient patient is at an increased hemolytic anemia and possibly death during rigorous exercise.

Works Cited

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