Pathological Analyses of Enzyme Deficiency in Human Erythrocyte Using E-Cell System

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1 Introduction

Human erythrocyte has been well-studied in last three decades, and extensive biochemical data on its enzymes and metabolites have been accumulated. Since the discovery of pyruvate kinase deficiency, erythroenzymopathies associated with hereditary hemolytic anemia have been extensively investigated.

Last year, we started to construct a simulation model of human erythrocyte. The first prototype of our cell model, which was completed in March of 1999, consists of the glycolysis pathway, the pentose phosphate pathway, and the nucleotide metabolism. Parameters of their kinetic equations are all based on experimental data found in the literature.

In this work, we obtained the steady state of the normal erythrocyte. The model was then modified in parameters to simulate enzyme deficiencies such as Pyruvate kinase (PK) deficiency.

2 An approach to calculate a steady state of simulation models using E-Cell System.

We examined several model variants which have alternative equations and parameters in order to obtain a concentration data set of the steady state. One of the simulation models we examined reached to the steady state (Table 1) after running for about 200,000 seconds in the simulation time, spending a little more than one week on a computer consisting of dual Compaq Alpha 21264 500MHz CPUs.

We are now trying to solve the set of simultaneous equations to obtain more accurate data set of metabolite concentrations at a steady state. The calculation is started with a minimum set of metabolic intermediate concentrations (ATP, Glucose, etc.) and full set of kinetic parameters that is based on the experimental analyses. Using these parameters, the activities of enzymes in the model are determined by solving the multiple equations. Concentrations of a steady state are calculated with these activities. We will adapt this method to the E-Cell system as a module of the simulation environment.

3 Simulation and analysis of Pyruvate kinase deficiency

PK is a key enzyme which produces ATP in the glycolysis pathway, and the mutation of the PK enzyme reduces its activity. We modified kinetic parameters of PK to fit for the three types of mutant, and the simulation experiments were carried out respectively with the steady state concentrations of the normal erythrocyte (Fig. 1). The amount of ATP was gradually reduced and eventually exhausted.

Table 1: Steady state concentrations of metabolic intermediates of Glycolysis.

Metabolic intermediate	ID	Initial value	Steady state	Experimental data
1,3-Diphosphogrycerate	13DPG	4.00×10^{-04}	1.83×10^{-04}	4.00×10^{-04}
2-Phosphogrycerate	2PG	1.40×10^{-02}	4.16×10^{-03}	1.40×10^{-02}
3-Phosphogrycerate	3PG	4.50×10^{-02}	4.62×10^{-02}	4.50×10^{-02}
Dihydroxy acetone phosphate	DHAP	1.40×10^{-01}	1.35×10^{-01}	1.40×10^{-01}
Fructose 6-phosphate	F6P	1.60×10^{-02}	6.39×10^{-02}	1.60×10^{-02}
Fructose 1,6-diphosphate	FDP	7.60×10^{-03}	1.14×10^{-02}	7.60×10^{-03}
Glucose 6-phosphate	G6P	3.80×10^{-02}	1.96×10^{-01}	3.80×10^{-02}
Glyceraldehyde 3-phosphate	GA3P	6.70×10^{-03}	6.24×10^{-03}	6.70×10^{-03}
Lactate	LACi	$1.10 \times 10^{+00}$	$1.20 \times 10^{+00}$	$1.10 \times 10^{+00}$
Phosphoenolpyruvate	PEP	1.70×10^{-02}	1.89×10^{-02}	1.70×10^{-02}
Pyruvate	PYRi	7.70×10^{-02}	6.00×10^{-02}	7.70×10^{-02}
2,3-Diphosphogrycerate	23DPG	$4.50 \times 10^{+00}$	$4.21 \times 10^{+00}$	$4.50 \times 10^{+00}$
Adenosine diphosphate	ADP	2.70×10^{-01}	2.20×10^{-01}	2.70×10^{-01}
Adenosine monophosphate	AMP	8.00×10^{-02}	2.42×10^{-02}	8.00×10^{-02}
Adenosine triphosphate	ATP	$1.54 \times 10^{+00}$	$1.57 \times 10^{+00}$	1.54×10^{-00}

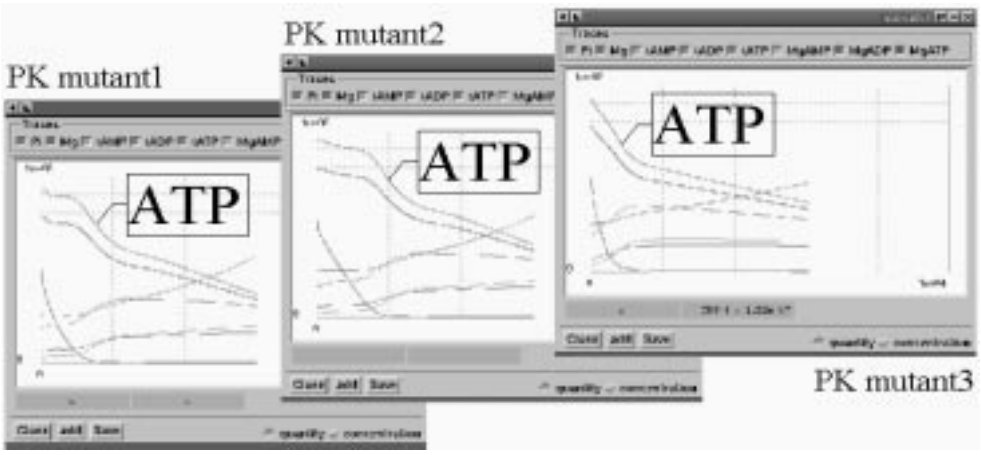

Figure 1: Effects of PK mutant on metabolic intermediates.

The longevity of our computer model in this experiments turned out to be much shorter than the real erythrocyte with PK mutation. The reason for this difference is presumably because the initial concentrations in this simulation were set too far from concentrations of the real PK mutant cell in the steady state. To solve this problem, we are currently trying to determine a steady state of PK mutant with the mathematical method described in the previous section.

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References

[1] Joshi, A. and Palsson, B.O., Metabolic dynamics in the human red cell, Part I-IV, *J. Theor. Biol.*, 141(4):515–528, 1989, 141(4):529-545, 1989, 142(1):41–68, 1990, 142(1):69–85, 1990.

[2] Jacobasch, G., Holzhutter, H., and Bisdorf, A., The energy metabolism of pyruvate kinase deficient red blood cells, *Haematologia (Budap)*, 17(2):259-266, 1984.