THERMODYNAMIC CONSIDERATIONS ON ERYTHROCYTE GLYCOLYSIS

Shigeki Minakami and Haruhisa Yoshikawa

Department of Physiological Chemistry and Nutrition,
Faculty of Medicine, University of Tokyo, Tokyo, Japan

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Human erythrocytes seemed to be one of the most suitable cells for the study of glycolysis in living cells, as neither tricarboxylic acid cycle nor respiration is present. Furthermore, hexose-monophosphate shunt contributes little to the break down of glucose in normal conditions and both protein and lipid syntheses could not be observed in the cells. Taking advantage of these characteristics of red cells and using the enzymatically determined concentrations of intermediates in red cells during steady-state glycolysis, the changes in the free-energies at the individual steps in erythrocyte glycolysis are calculated.

The free energy changes associated with the individual steps of glycolysis have been calculated by Burton and Krebs (1953), though the lack of data at that time forced them to calculate the energy changes on the assumption that the concentrations of all reactants to be 10 mM. The advantages of the present calculation are 1) the data are based on the actual concentrations of the reactants in the cells and 2) there exists little interaction between glycolysis and other metabolic systems, such as respiration, hexose-monophosphate shunt and protein synthesis.

The data employed for the calculation.----- The data for the intermediates and coenzymes are from the previous communication (Minakami, Saito, Suzuki

Abbreviations. ΔF: free energy change under specified conditions, $\Delta F'$: free energy change under standard conditions except H^+ ion concentration is specified, Gluc: glucose, G6P: glucose 6-phosphate, F6P: fructose 6-phosphate, FDP: fructose diphosphate, GAP: glyceraldehyde phosphate, DHAP: dihydroxyacetone phosphate, 2,3DPG: 2,3-diphosphoglycerate, 1,3DPG: 1,3-diphosphoglycerate, 3PGA: 3-phosphoglycerate, 2PGA: 2-phosphoglycerate, GA: glycerate, PEP: phosphoenolpyruvate, Pyr: pyruvate, Lact: lactate, Pi: inorganic phosphate.

and Yoshikawa, 1964), namely, glucose 5 mM, G6P 83 μM, F6P 14 μM, FDP 31 μM, DHAP 138 μM, GAP 18.5 μM, 3PGA 118 μM, 2PGA 29.5 μM, PEP 23 μM, 2,3DPG 6.85 mM, ATP 1.85 mM, ADP 0.138 mM, Pyr 51 μM, Lact 2.9 mM and Pi 1 mM. Assumptions that glucose, lactate and pyruvate are evenly distributed between the cells and plasma, and that water content inside the cells is 0.7 ml/ml cells, are used for the calculations. The concentrations of inorganic phosphate in the media was taken as representing the Pi in the reaction.

The changes of free energies at the individual steps of glycolysis.----- Table I shows the changes of free energies calculated from the steady-state concentrations of the intermediates and the coenzymes in red cells. The calculations are based on the standard free energy data of Burton (1957) at pH 7.0, 25° and from the equation $\Delta F = \Delta F' + 1.42 \log K$. The changes of free energies due to temperature (25° and 37°) are neglected, as they are not so large (Huennekens and Whiteley, 1960). The ratio [NAD]/[$NADH_2$] is calculated to be 240 from the ratio [Pyr]/[Lact] and equilibrium constant 4.4×10^{-12} (Racker, 1950), assuming pyruvate and lactate are in equilibrium. The data are also graphically shown in Fig. 1.

Table I.

Free energy changes for the individual steps of erythrocyte glycolysis

	ΔF	$(\Delta F')$*
Hexokinase	-8.0	(-5.1)
Glucosephosphate isomerase	-0.61	(0.5)
Phosphofructokinase	-5.3	(-4.2)
Aldolase	-0.29	(5.51)
Triosephosphate isomerase	0.59	(1.83)
Glyceraldehydephosphate dehydrogenase + Phosphoglycerate kinase	0.37	(-3.25)
Phosphoglyceromutase	0.18	(1.06)
Enolase (Phosphopyruvate hydratase)	-0.80	(-0.64)
Pyruvate kinase	-4.0	(-6.1)
Lactate dehydrogenase	0 **	(-6.0)

* $\Delta F'$: for 25°, pH 7.0 (Burton, 1957)
** assumed

Discussions on the rate-limiting steps in erythrocyte glycolysis.----- If the activity of an enzyme in a glycolytic system is fast enough compared to

the overall rate of glycolysis, the equilibrium constant calculated from $\Delta F'$ should be close to the corresponding ratio calculated from the steady-state concentrations of the intermediates and accordingly ΔF should be small. From Table I, it may be considered that glucosephosphate isomerase, aldolase, triosephosphate isomerase, glyceraldehydephosphate dehydrogenase, phosphoglycerate kinase, phosphoglyceromutase and enolase could not be rate-limiting in erythrocyte glycolysis. Conversely, as the free energy changes at three steps, namely, hexokinase, phosphofructokinase and pyruvate kinase, are large and the reactants are far from thermodynamic equilibrium, these three steps may

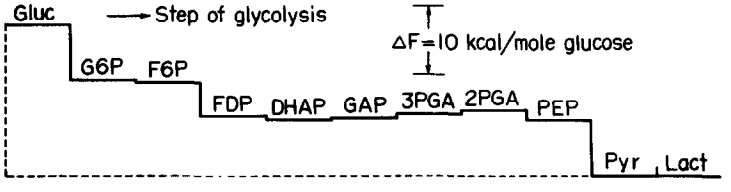

Fig. 1. Free energy changes in erythrocyte glycolysis

be the rate-limiting steps in erythrocyte glycolysis and the change of the reaction rate at one of these steps will cause the change of over-all glycolytic rate.

The increase of lactate formation in erythrocyte by increasing pH has been shown to be due to the increase of phosphofructokinase activity (Minakami, Saito, Suzuki and Yoshikawa, 1964) and the effect of ouabain on erythrocyte glycolysis is suggested to be due to the inhibition of the phosphofructokinase step (Minakami, Kakinuma and Yoshikawa, 1964). Numerous other examples of the controlling nature of these steps, especially, of phosphofructokinase, have been reported with regard to other kind of cells.

1,3-Diphosphoglycerate and 2,3-diphosphoglycerate.----- Though 1,3-DPG could not be measured due to low concentration and extreme lability, it may be calculated by assuming it to be equilibrated with GAP and 3PGA. This could

be justified as GAP and 3PGA are close to thermodynamic equilibrium. It is estimated to be 0.39 μM from the equilibrium of glyceraldehydephosphate dehydrogenase and 0.7 μM from that of phosphoglycerate kinase.

The concentration of 2,3DPG in erythrocytes is known to be extremely high compared to other cells and the possibility of the pathway 1,3DPG → 2,3DPG → 3PGA + Pi has been suggested. The free energy changes for 1,3DPG → 2,3DPG and 2,3DPG → 3PGA + Pi in erythrocytes are calculated to be - 4.1 kcal and -6.5 kcal, respectively, from the standard free energy data for the hydrolysis of 1,3DPG and 2PGA (14.3 kcal from Burton, 1961, and 4 kcal from Atkinson and Morton, 1961, respectively) and 1,3DPG concentration of 0.5 μM. The identity of free energy changes for 2PGA → GA + Pi and 2,3DPG → 3PGA + Pi is assumed. This shunt, however, may be operating in steady-state glycolysis of the cells in limited extent* and may be disregarded, as the rate of P^{32} incorporation into 2,3DPG is several times smaller than into ATP (Tatibana et al., 1960).

The efficiency of ATP formation by erythrocyte glycolysis.----- The free energy change of ATP hydrolysis in red cells is calculated to be -13.3 kcal from the standard free energy change of -8.9 kcal (at pH 7.5, Burton, 1957) and the concentrations of ATP, ADP and Pi. The free energy change for the erythrocyte glycolysis may be calculated directly from the standard free energy (-47.4 kcal, Burton, 1957) and the concentrations of glucose and lactate as -51 kcal. It can also be calculated to be -49 kcal from the sum of free energy changes (Table I) and ΔF for ATP hydrolysis. The efficiency of ATP formation by the steady-state glycolysis of erythrocyte is thus calculated to be 26.6/50 = 53 %.

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* The slight increase of the free energy from GAP to 3PGA in Table I might reflect the slight contribution of the shunt without the production of ATP.

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