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FREE ENERGY DISSIPATION OF THE PYRUVATE KINASE REACTION HAS A MINIMUM AT CELL METABOLITE CONCENTRATIONS

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The ratio of substrates and products (mass action ratio) for the reaction catalyzed by the enzyme pyruvate kinase is measured under the constraint of constant reaction rate for pyruvate kinase (EC 2.7.1.40) from brewers yeast and *Escherichia coli*. For both organisms, a maximum of the ratio is found at concentrations comparable to those obtained from cell metabolite measurements. This observation suggests an optimum principle for free energy transduction in the glycolytic reaction pathway, as a maximum of the mass action ratio corresponds to a minimum dissipation of free energy.

1. Introduction

The rate of glycolysis has a constant value at least for definite time intervals in the life cycle of the cell. This constraint of a steady rate can be met by a broad range of metabolite concentrations at each enzyme-catalyzed step, because most of the enzymic rates are regulated by more than one metabolite. We consider here the reaction step catalyzed by the enzyme pyruvate kinase (EC 2.7.1.40):

$$\text{Pyr-P} + \text{ADP} \rightleftarrows \text{Pyr} + \text{ATP}$$

The rate of the chemical turnover depends most significantly on five ligands, if the quasi-steady-state assumption is made:

$$v = V/V_{max} = v([\text{Pyr-P}], [\text{ADP}], [\text{ATP}], [\text{FBP}], [\text{Mg}^{2+}]) \quad (1)$$

The dependence of the reaction rate on pyruvate can be neglected and the concentration of pyruvate is set to 1 mM for all calculations. The constraint of a steady-state flux $v = v_{ss}$ defines at constant [FBP] and $[Mg^{2+}]$ a surface in the three-dimensional concentration space of [Pyr-P], [ADP] and [ATP]. The mass action ratio

$$K_{ss} = \frac{[\text{ATP}][\text{Pyr}]}{[\text{ADP}][\text{Pyr-P}]} \quad (2)$$

varies on this surface and the functional relation between metabolite concentrations and rate as given by eq. 1 may lead to extremum properties of K_{ss}.

Extrema of K_{ss} are determined by the equations

$$\frac{\partial \ln[\text{Pyr-P}]}{\partial \ln[\text{ATP}]} = +1 \quad (3)$$

$$\frac{\partial \ln[\text{Pyr-P}]}{\partial \ln[\text{ADP}]} = -1 \quad (4)$$

These equations are derived by equating to zero the partial derivatives of $\ln K_{ss}$ with respect to ln[ATP] and ln[ADP], assuming that [Pyr-P] is a single-valued function of [ATP] and [ADP] on the surface of constant rate. The geometrical interpretation of an extremum in K_{ss} is shown in fig. 1 with a rate law for pyruvate kinase from *Sac-*

Abbreviations: Pyr, pyruvate; Pyr-P, phospho*enol*pyruvate; FBP, fructose 1,6-bisphosphate; v, reaction rate normalized with respect to its maximum value V_{max}.

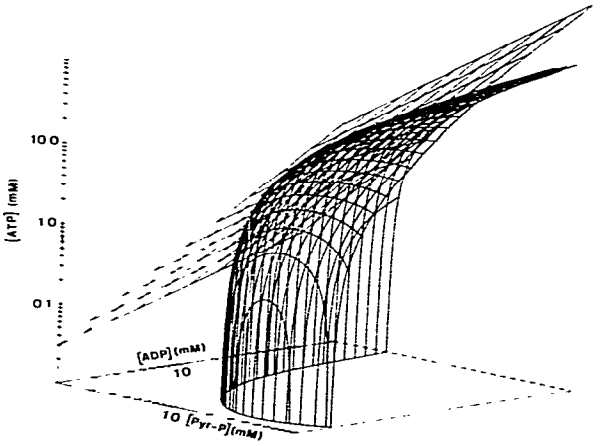

Fig. 1. Rate surface for $v_{ss} = 0.4$. The surface is tangentially touched by the plane, $\ln[ATP] - \ln[ADP] - \ln[Pyr\text{-}P] = \ln(K_{ss})_{max}$, at the point where the steady-state mass action ratio is maximum.

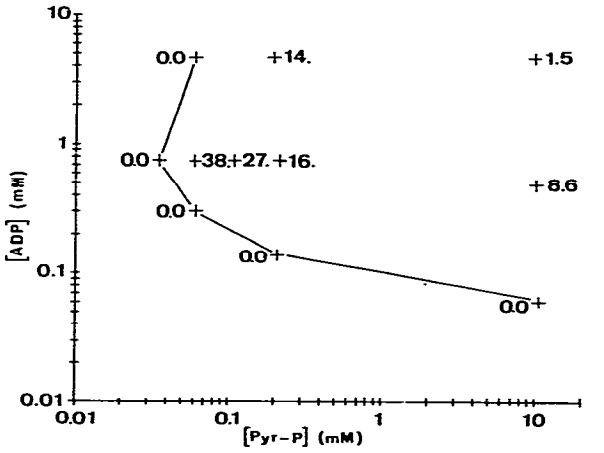

Fig. 2. Pyruvate kinase from *E. coli*: K_{ss} values determined by iterative initial rate experiments for $v_{ss} = 0.06$. [FBP] = 5 mM, $[Mg^{2+}] = 1$ mM, pH = 7.0, $T = 25°C$, ionic strength = 300 mM.

charomyces carlsbergensis valid for high FBP concentrations (see below). The surface of constant rate $v = v_{ss}$ is tangentially touched by the plane

$$\ln[ATP] - \ln[ADP] - \ln[Pyr\text{-}P] = \ln K_{ss} \quad (5)$$

at the point where K_{ss} is extremum. The extremum is a maximum due to the convexity of the surface $v = v_{ss}$.

2. Results

The existence of a K_{ss} maximum for a given steady state was verified by initial rate experiments. The results for pyruvate kinase from *Escherichia coli* and from *S. carlsbergensis* are shown in figs. 2 and 3, respectively. Each cross in these figures was determined by a series of measurements holding [ADP] and [Pyr-P] constant. In this series of measurements we changed [ATP] step by step (an average of five measurements was necessary) until the constraint of given $v = v_{ss}$ was met. K_{ss} was then calculated by eq. 2 and is shown by the number at the side of the crosses in figs. 2 and 3. The line linking the points marked 0.0 is a rough interpolation, indicating the curve where the absence of the non-essential effector ATP allows the fulfillment of $v = v_{ss}$, implying a zero mass action ratio.

The maximum in fig. 2 corresponds to [Pyr-P]

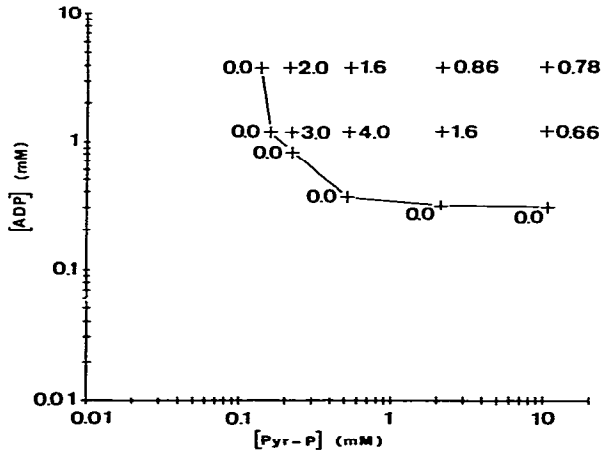

Fig. 3. Pyruvate kinase from brewers yeast: K_{ss} values determined by iterative initial rate experiments for $v_{ss} = 0.4$, [FBP] = 15 mM, $[Mg^{2+}] = 2$ mM, pH = 6.0, $T = 25°C$, ionic strength = 300 mM.

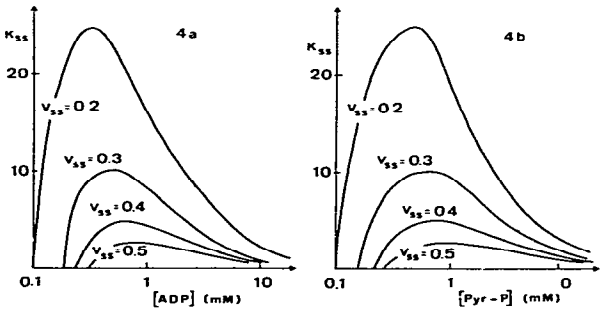

Fig. 4. Steady-state mass action ratio K_{ss} calculated with a rate law for pyruvate kinase from brewers yeast. [FBP] = 15 mM, $[Mg^{2+}]$ = 2 mM, [Pyr] = 1 mM, pH = 6.0, T = 25°C, ionic strength = 300 mM. [Pyr-P] ([ADP]) was kept constant in each of the curves in panel a (b) at the value where the K_{ss} surface defined by $v = v_{ss}$ reached its maximum.

= 0.06 mM, [ADP] = 0.75 mM and [ATP] = 1.7 mM ([FBP] = 5.0 mM as experimental condition). Metabolite measurements by Lowry et al. [1] for *E. coli* cells yield comparable concentrations: [Pyr-P] = 0.08 mM, [ADP] = 0.8 mM and [ATP] = 2.4 mM ([FBP] = 2.6 mM; a water content of 2.5 ml/g dry weight is assumed). The maximum in fig. 3 corresponds to [Pyr-P] = 0.5 mM, [ADP] = 1.2 mM and [ATP] = 2.4 mM ([FBP] = 15 mM). Measurements by Hess et al. [2] on yeast extract oscillating around a steady state yield also comparable concentrations: [Pyr-P] = 0.2–0.9 mM, [ADP] = 0.5–1.4 mM and [ATP] = 1.0–2.1 mM ([FBP] = 18 mM).

In order to refine this investigation, a further 119 initial rate experiments were carried out with pyruvate kinase from *S. carlsbergensis* (M. Markus, T. Plesser, A. Boiteux and B. Hess, unpublished data). A rate law analogous to that described in ref. 3 was fitted to these data. Fig. 4 shows K_{ss} calculated from this rate law for different v_{ss}. In this refined evaluation, the position of the maximum of K_{ss} for $v_{ss} = 0.4$ is somewhat displaced with respect to that in fig. 3 owing to interpolation by the model.

3. Discussion

Our experimental findings are correlated with thermodynamic properties of the system in the following manner.

The mass action ratio K_{ss} is connected to the dissipation of free energy by the dissipation function ϕ_{ss}, which is given by [4].

$$\phi_{ss} = -v_{ss}V_{max}\Delta G = v_{ss}V_{max}A_{PK} = v_{ss}V_{max}RT\{\ln K - \ln K_{ss}\} > 0 \quad (6)$$

where A_{PK} is the affinity and K the equilibrium constant of the pyruvate kinase reaction. A maximum in K_{ss} for a given constant rate v_{ss} thus means a minimum of dissipation of free energy. Therefore, the observed maximum in K_{ss} may be correlated with optimal free energy transduction and efficiency [4–8]. The minimum in ϕ_{ss} described here must not be associated with the principle of minimum entropy production [9], because the former is a minimum within a continuum of steady states and not a minimum with respect to a deviation from a steady state.

From the free energy profile of the glycolytic pathway [10] it is known that the phosphofructokinase and the pyruvate kinase reactions are in a state far from equilibrium. To a first approximation, it is reasonable to assume that the reaction sequence between [FBP] and [Pyr-P] is only slightly displaced from equilibrium. This allows the following relation for the total glycolytic dissipation function:

$$\phi_{ss} = v_{ss}(\tfrac{1}{2}A_{PFK} + A_{PK}) \quad (7)$$

where A_{PFK} is the affinity of the phosphofructokinase reaction. The steady-state rate refers to pyruvate kinase. Since both A_{PFK} and A_{PK} are positive, it is reasonable to argue that both enzymes are optimized with respect to their contribution to free energy dissipation.

Our findings suggest that the maximization of the steady-state mass action ratio is a significant regulatory aim of cell metabolism. Since the metabolite concentrations at the maximum of K_{ss} are determined by the corresponding binding constants of the metabolites on the enzyme, this effect must be the result of an evolutionary process

designing the properties of the enzymes.

All considerations presented in this work may easily be adapted to enzymic reactions in other metabolic pathways.

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References

1 O.H. Lowry, J. Carter, J.B. Word and L. Glaser, J. Biol. Chem. 246 (1971) 6511.
2 B. Hess, A. Boiteux and H. Krüger, Adv. Enzyme Regul. 7 (1969) 149.
3 M. Markus, T. Plesser, A. Boiteux, B. Hess and M. Malcovati, Biochem. J. 189 (1980) 421.
4 S.R. Caplan, Curr. Top. Bioenerg. 4 (1971) 1.
5 T. Bücher and W. Rüssmann, Angew. Chem. 19 (1963) 881.
6 B. Hess, in: Energy transformation in biological systems. No. 31 (Ciba Foundation Symposium) (North-Holland, New York, 1975) p. 369.
7 P.H. Richter and J. Ross, Biophys. Chem. 12 (1980) 285.
8 T. Plesser and M. Markus, Hoppe-Seyler's Z. Physiol. Chem. 363 (1982) 546.
9 I. Prigogine, Introduction to thermodynamics of irreversible processes (John Wiley, New York, 1967).
10 A. Lehninger, Biochemistry (Worth Publishers, Inc., New York, 1971) 3rd printing, p. 327.