*Proc. Natl. Acad. Sci. USA*
Vol. 78, No. 6, pp. 3563–3566, June 1981
Biophysics

# Oscillations and control features in glycolysis: Analysis of resonance effects

(phosphofructokinase–pyruvate kinase reaction coupling/efficiency/ATP/ADP ratio)

YVES TERMONIA AND JOHN ROSS

Department of Chemistry, Stanford University, Stanford, California 94305

*Contributed by John Ross, February 12, 1981*

**ABSTRACT** **We have presented [Termonia, Y. & Ross, J. (1981) *Proc. Natl. Acad. Sci. USA* 78, 2952–2956] an analysis of glycolysis based on experimental findings. Here, we give an interpretation based on the concepts of efficiency, resonance response, and control features available to highly nonlinear reaction kinetics. The comprehensive model of glycolysis, for which we presented numerical evaluation of concentration oscillations, entropy production, and average ATP/ADP ratios is separated into two subsystems: one for the phosphofructokinase (PFKase) reaction, the other for the pyruvate kinase (PKase) reaction. We analyze each subsystem separately and find that, for a range of parameter values around the best estimates obtained from experimental data, the PFKase reaction exhibits sustained oscillations. The PKase reaction, on the other hand, is in a stable stationary state and shows an oscillatory relaxation. The period of the PFKase reaction is within 25% of that obtained by computer simulation of the full model. Moreover, if, for a given set of kinetic parameters, the period of the oscillations in the PFKase reaction is of the same order of magnitude as the period of the oscillatory relaxation of the PKase reaction, then the onset of oscillatory behavior past marginal stability forces a tuning of the PFKase reaction period to that of the PKase reaction. Thus, the PKase reaction tunes the frequency of the primary oscillophor, the PFKase reaction, so that a resonance response results in the PKase reaction. We find this result for a substantial range of parameters. The sharp increase in the ATP/ADP ratio and the decrease in the dissipation (entropy production) are seen to be a result of the forced tuning to resonance. The origin of the remarkable control feature of the tuning of one part of the pathway by another that follows in the reaction mechanism is the coupling of the two parts and, in the case of glycolysis, this is realized by the intermediate fructose 1,6-diphosphate, which participates crucially in the PFKase and PKase reactions.**

We have proposed (1) a comprehensive model for the glycolytic reaction mechanism, including an important coupling of the phosphofructokinase (PFKase) and pyruvate kinase (PKase) reactions. With the model, we calculated concentrations of chemical intermediates and found oscillatory behavior over a range of kinetic parameters, including the best estimates obtained from experiment. In addition, we calculated the ATP/ADP ratio and the free energy dissipation (entropy production). We found that both quantities undergo an abrupt change past the onset of chemical oscillation.

In previous work (2–4), the possibility of resonance response of certain reaction mechanisms, including oscillatory ones, has been investigated. When an oscillatory reaction is driven by external concentration variations, then, at certain frequencies, a resonance response can occur and manifest itself in alterations of the free energy dissipation, depending on the phase of the driving frequency compared with that of the oscillatory reaction. Both increases and decreases in dissipation may occur in a very narrow range of the driving frequency, which points to possible regulatory functions. As the dissipation is decreased, the efficiency of the reaction is increased. It has been suggested (3) that the possibility of such control features may have contributed to the evolutionary development of oscillatory biochemical reaction mechanisms.

The purpose of this article is to determine whether these and other control features play a role in glycolysis, as represented by the model studied in ref. 1. In this analysis, we first need to check, in agreement with previous work (for review, see ref. 5), that the PFKase reaction is the primary oscillophor in the glycolytic pathway. We then need to inquire about the relationship between the PFKase and the PKase reactions. To do this, we have to divide the system into two parts and recognize that simplification is justified if the results are nearly the same as those for the full model and if it leads to insight. We analyze each subsystem separately and find that, for a range of parameter values around the best estimates obtained from experimental data, the PFKase reaction exhibits self-sustained oscillations. The PKase reaction, on the other hand, shows an oscillatory relaxation in that, on perturbation, the reaction returns to its stable stationary state with oscillatory kinetics. The first justification for the simplified analysis is the fact that the period of the PFKase reaction, for the range of parameters used, is within 25% of that obtained by a computer simulation of the full model. The second justification is the following insight: If the period of oscillation in the PFKase reaction is of the same order of magnitude as the period of oscillatory relaxation of the PKase reaction, below or at marginal stability, then the onset of oscillatory behavior past marginal stability forces a tuning of the PFKase reaction period to that of the PKase (i.e., it forces a resonance at the fundamental frequency). We find this result for a substantial range of parameters; hence, it is not an accidental result of the choice of one set of parameters. The sharp increase in the ATP/ADP ratio and the decrease in the dissipation found in ref. 1 are seen to be a result of the forced tuning of the PFKase reaction by the PKase reaction and the consequent resonance response of the PKase reaction to the primary oscillophor, the PFKase reaction.

We next address the question of the origin of the remarkable control feature of the tuning of one part of the pathway by another, which follows in the reaction mechanism. It is, of course, the coupling of the two systems that brings this about and, in the case of glycolysis, this is realized by the intermediate fructose 1,6-diphosphate (Fru-1,6-$P_2$), which participates crucially

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: Fru-6-*P*, fructose 6-phosphate; Fru-1,6-$P_2$, fructose 1,6-diphosphate; *P*-*e*Prv, phospho*enol*pyruvate; PFKase, phosphofructokinase; PKase, pyruvate kinase; A(MDT)P, total adenine nucleotides.

in the PFKase and PKase reactions. Decoupling has been done in all previous models (5), and it leads to destruction of tuning and hence resonance response.

## SIMPLIFIED ANALYSIS

The notation of ref. 1 is used below; equations and figures of that paper are designated by the prefix I.

In this section, we present an approximate analysis that permits the study of the oscillatory behavior of the PFKase and of the PKase reactions separately. The objective is the decomposition of the model into two coupled two-variable subsystems (one for PFKase and one for PKase) and a linear stability analysis of each of them. Our full model (see figure I-1) has four essential variables—the concentrations of fructose 6-phosphate (Fru-6-*P*), Fru-1,6-$P_2$, phospho*enol*pyruvate (*P*-*e*Prv), and ATP. The concentration of pyruvate is not essential because it does not affect the kinetics of the PFKase and PKase reaction steps and those of ADP and of AMP can be expressed as functions of the ATP concentration by using the adenylate kinase reaction and the conservation of total adenine nucleotide concentration (see Eqs. **I-15** and **I-16**). Among the four variables cited above, two interact with the key enzymes PFKase and PKase: Fru-1,6-$P_2$ controls PKase, and ATP is the only metabolite that controls PFKase. Thus, the two variables necessary to describe the PFKase subsystem are ATP and Fru-1,6-$P_2$, whereas those for the PKase subsystem are Fru-1,6-$P_2$ and *P*-*e*Prv, the two subsystems being coupled through the variable Fru-1,6-$P_2$.

**Linearization of the PFKase Subsystem.** Linearization of the total time derivatives of the Fru-1,6-$P_2$ and ATP concentrations, with the concentrations of Fru-6-*P* and *P*-*e*Prv held constant, leads to the relaxation matrix

$$\Lambda_{\text{PFKase}} = \begin{pmatrix} \dfrac{\partial[\dot{\text{Fru-1,6-}}P_2]}{\partial[\text{Fru-1,6-}P_2]} & \dfrac{\partial[\dot{\text{Fru-1,6-}}P_2]}{\partial[\text{ATP}]} \\ \dfrac{\partial[\dot{\text{ATP}}]}{\partial[\text{Fru-1,6-}P_2]} & \dfrac{\partial[\dot{\text{ATP}}]}{\partial[\text{ATP}]} \end{pmatrix}, \qquad [1]$$

with (see Eqs. **I-11** and **I-14**)

$$\frac{\partial[\dot{\text{Fru-1,6-}}P_2]}{\partial[\text{Fru-1,6-}P_2]} = -\frac{\partial V_3}{\partial[\text{Fru-1,6-}P_2]} \qquad [2]$$

$$\frac{\partial[\dot{\text{Fru-1,6-}}P_2]}{\partial[\text{ATP}]} = \frac{\partial V_2}{\partial[\text{ATP}]} \qquad [3]$$

$$\frac{\partial[\dot{\text{ATP}}]}{\partial[\text{Fru-1,6-}P_2]} = 2\frac{\partial V_3}{\partial[\text{Fru-1,6-}P_2]} + \frac{\partial V_4}{\partial[\text{Fru-1,6-}P_2]} \qquad [4]$$

$$\frac{\partial[\dot{\text{ATP}}]}{\partial[\text{ATP}]} = -\frac{\partial V_2}{\partial[\text{ATP}]} + \frac{\partial V_4}{\partial[\text{ATP}]} - \frac{\partial V_6}{\partial[\text{ATP}]}. \qquad [5]$$

The partial derivatives of the flows are given in the *Appendix*. Inspection of the eigenvalues of that matrix shows that there exists, in the parameter space, a region in which self-sustained oscillations may appear.

The only positive contribution in the trace $[(\partial[\dot{\text{Fru-1,6-}}P_2]/\partial[\text{Fru-1,6-}P_2]) + (\partial[\dot{\text{ATP}}]/\partial[\text{ATP}])]$ of the matrix $\Lambda_{\text{PFKase}}$ comes from the term $(-\partial V_2/\partial[\text{ATP}])$, which is always positive [see Eq. **A1**, where, for our choice $K = 1$ (1), $(\partial[\text{AMP}]/\partial[\text{ATP}]) < 0$]. Inspection of Eq. **A1** also shows that $(\partial V_2/\partial[\text{ATP}]) \to 0$ as the concentration of Fru-6-*P* becomes either very small ($[\text{Fru-6-}P] \to 0$) or very large ($[\text{Fru-6-}P] \to \infty$). Thus, there is a finite domain of substrate input for which self-sustained oscillations may occur. That observation is in agreement with experimental findings (6).

**Linearization of the PKase Subsystem.** Linearization of the total time derivatives of the Fru-1,6-$P_2$ and *P*-*e*Prv concentrations, with the concentrations in Fru-6-*P* and ATP held constant, leads to the relaxation matrix

$$\Lambda_{\text{PKase}} = \begin{pmatrix} \dfrac{\partial[\dot{\text{Fru-1,6-}}P_2]}{\partial[\text{Fru-1,6-}P_2]} & \dfrac{\partial[\dot{\text{Fru-1,6-}}P_2]}{\partial[P\text{-}e\text{Prv}]} \\ \dfrac{\partial[P\text{-}e\dot{\text{P}}\text{rv}]}{\partial[\text{Fru-1,6-}P_2]} & \dfrac{\partial[P\text{-}e\dot{\text{P}}\text{rv}]}{\partial[P\text{-}e\text{Prv}]} \end{pmatrix} \qquad [6]$$

with (see Eqs. **I-11** and **I-12**)

$$\frac{\partial[\dot{\text{Fru-1,6-}}P_2]}{\partial[\text{Fru-1,6-}P_2]} = -\frac{\partial V_3}{\partial[\text{Fru-1,6-}P_2]} \qquad [7]$$

$$\frac{\partial[\dot{\text{Fru-1,6-}}P_2]}{\partial[P\text{-}e\text{Prv}]} = -\frac{\partial V_3}{\partial[P\text{-}e\text{Prv}]} \qquad [8]$$

$$\frac{\partial[P\text{-}e\dot{\text{P}}\text{rv}]}{\partial[\text{Fru-1,6-}P_2]} = 2\frac{\partial V_3}{\partial[\text{Fru-1,6-}P_2]} - \frac{\partial V_4}{\partial[\text{Fru-1,6-}P_2]} \qquad [9]$$

$$\frac{\partial[P\text{-}e\dot{\text{P}}\text{rv}]}{\partial[P\text{-}e\text{Prv}]} = 2\frac{\partial V_3}{\partial[P\text{-}e\text{Prv}]} - \frac{\partial V_4}{\partial[P\text{-}e\text{Prv}]}. \qquad [10]$$

The partial derivatives of the flows are given in the *Appendix*. Inspection of the eigenvalues of that matrix shows that there is the possibility of oscillations, which can only be damped. The presence of a reverse reaction in step 3—i.e. $\bar{k}_3 \neq 0$—is essential for the occurrence of oscillatory relaxation kinetics in the PKase reaction.

We want to stress that this approximate analysis does not preclude the possibility of self-sustained oscillations in the PKase reaction. Dynnik and Selkov (7), using computer simulation, have observed self-sustained oscillations in a three-variable model system for the later steps of the glycolytic pathway.

As far as we could determine, and for any choice of values for the parameters $k_3$, $\alpha$, $\bar{k}_3$, and $\beta$ that satisfy the experimental

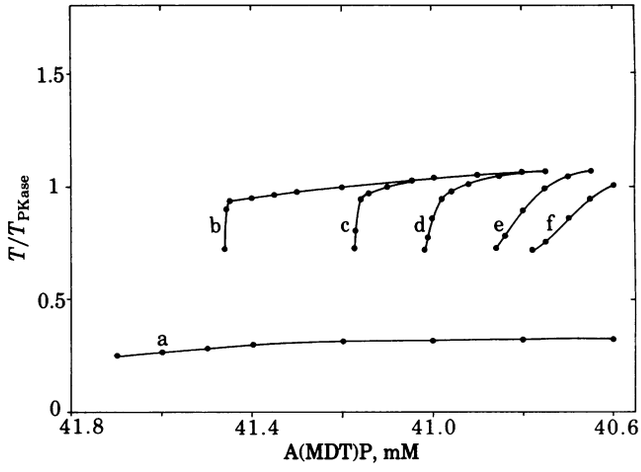


FIG. 1. Dependence of $T/T_{\text{PKase}}$ on A(MDT)P, where $T$ represents the period of the self-sustained oscillations obtained by computer simulation of our full model of the glycolytic pathway (see ref. 1), $T_{\text{PKase}}$ is the period of the damped oscillations in the PKase reaction as obtained from our simplified analysis (i.e., Eqs. **6–10**). The points are calculated, and the curve is drawn to connect the points. The figure is for different values of $k_3$, which represents the forward rate constant for step 3. The value $k_3 = 5.8$ is that suggested from experimental findings (1). The values of the other parameters are those given in table 1 of ref. 1. The points of marginal stability are the ones on the extreme left of each curve. Curves: a, $k_3 = 2.2$; b, $k_3 = 4$; c, $k_3 = 5$; d, $k_3 = 5.8$; e, $k_3 = 7$; f, $k_3 = 8$.

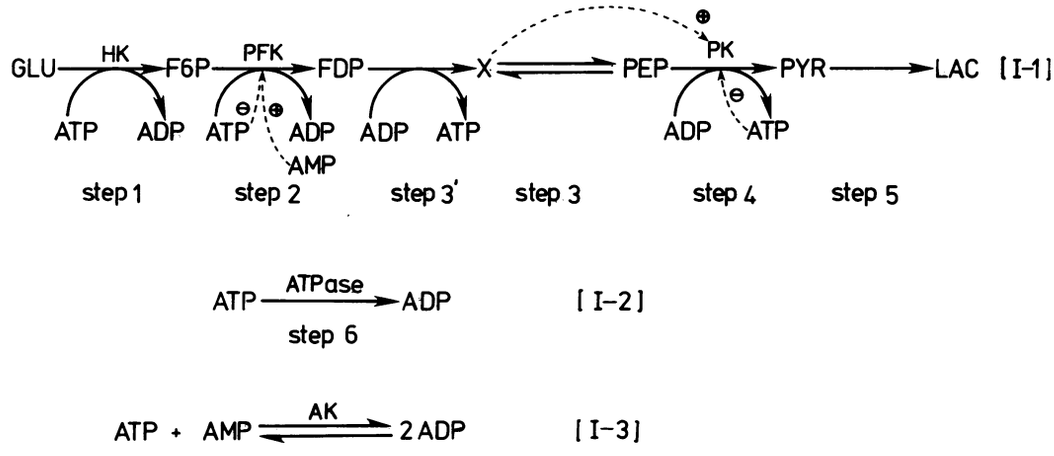


FIG. 2. Modified scheme for the glycolytic pathway, in which the PFKase and the PKase reactions are decoupled. X is a hypothetical metabolite. Arrows in one direction only indicate almost irreversible reactions. Arrows in both directions indicate reactions close to equilibrium. Broken lines indicate activations (⊕) or inhibitions (⊖) of enzymes by metabolites, which are taken into account in the model. GLU, glucose; HK, hexokinase; F6P, Fru-6-*P*; PFK, PFKase; FDP, Fru-1,6-$P_2$; PEP, *P*-*e*Prv; PYR, pyruvate; LAC, lactic acid; AK, adenylate kinase.

constraints (see ref. 1), our linear stability analysis predicts that self-sustained oscillations in the PFKase reaction are always associated with damped oscillations in the PKase reaction.

## RESULTS AND DISCUSSION

In this section, we justify the simplified analysis presented above by first comparing our results with those obtained in ref. 1 by a computer simulation of the full model. Further justification of the analysis is given by showing the insight one gains in the understanding of the results presented in ref. 1.

Results obtained from our linear stability analysis of the PFKase subsystem (i.e., by using Eqs. **1–5**) show that sustained oscillations are indeed expected in the range of parameter values investigated in figures I-3–I-5. In addition, the period $T_{PFKase}$ of the oscillations obtained from our analysis is within 25% of the period $T$ obtained by computer simulation of the full model (see figure I-3). Thus, in agreement with previous work (5), our analysis clearly indicates that the PFKase reaction is the primary oscillophor in the glycolytic pathway.

A second justification of our simplified analysis is provided by the following. Let $T_{PKase}$ denote the period of the damped oscillations in the PKase reaction, as obtained from our linear analysis (i.e., by using Eqs. **6–10**). Normalization by $T_{PKase}$ of the $T$ curves in figure I-3 leads to the curves shown in Fig. 1. Since, for given $k_3$, $T_{PKase}$ is constant and independent of the total adenine nucleotide concentration [A(MDT)P], the curves $T/T_{PKase}$ still essentially reflect the dependence of $T$ on A(MDT)P. Inspection of Fig. 1 shows that, for low $k_3$ ($k_3 = 2.2$, see curve a), $T$ is much less than $T_{PKase}$ ($T/T_{PKase} \approx 0.25$) and, as the distance from marginal stability is increased, $T$ remains almost invariant, whereas the oscillations increase in amplitude. For larger values of $k_3$, however, our simplified analysis shows that the periods of the PFKase and PKase oscillators become closer; a typical ratio $T/T_{PKase}$ of $\approx 0.715$ is observed at marginal stability. As the distance from marginal stability is slightly increased, the transitions already observed in figure I-3 are seen to bring the period $T$ very close to $T_{PKase}$. The curves subsequently level off at an almost invariant value close to $T_{PKase}$. Further investigation shows that, in the absence of damped oscillations in the PKase reaction (that situation can be obtained, for example, by decreasing the degree of activation of PKase by Fru-1,6-$P_2$), any value of $k_3$ ($2.2 \leq k_3 \leq 8$) leads to a $T$ curve similar to curve a. We also note that transitions similar to those observed in Fig. 1 have been obtained for a substantial range of parameters. Hence, the results are not accidental. Thus, the sharp increase in the ATP/ADP ratio and the decrease in the dissipation immediately after the onset of oscillations (1) are seen to be a result of the forced tuning of the PFKase reaction by the PKase reaction—the frequency of the primary oscillophor, the PFKase reaction, is tuned by the PKase reaction to resonance, which then allows control of dissipation and higher efficiency. We did not find any region in the [$k_3$, $\alpha$, $\bar{k}_3$, $\beta$] parameter space for which $T > T_{PKase}$ at marginal stability.

We now address the question of the origin of the remarkable control feature of the tuning of one part of the pathway by another that follows in the reaction mechanism. In Fig. 2, we modify the reaction sequence I-1 of ref. 1 by decoupling the PFKase and PKase reactions. This is realized by interposing between the two reactions an artificial step 3′ that produces,

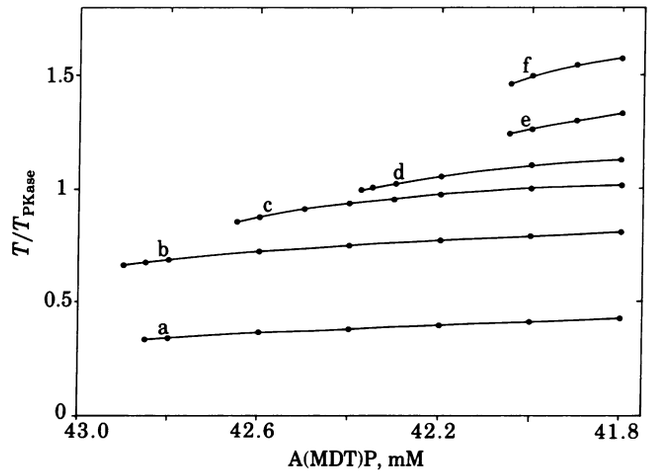


FIG. 3. Dependence of $T/T_{PKase}$ on A(MDT)P in the modified scheme for the glycolytic pathway, in which the PFKase and PKase reactions are decoupled (see Fig. 2). $T_{PKase}$ is the period of the damped oscillations in the PKase reaction, as obtained by a simplified analysis similar to that used for Fig. 1 but involving the variables *X* and *P*-*e*Prv. The points are calculated, and the curve is drawn to connect the points. The figure is for different values of $k_3$. The values of the other parameters are those used in Fig. 1, except for $\alpha = 0.2$, $\beta = 3$, $k_T^{ATP} = 0.186$ mM, and $\gamma = 4$. We took $k_3' = 0.5$ and $\alpha' = 2$. Curves: a, $k_3 = 4.5$; b, $k_3 = 7$; c, $k_3 = 10$; d, $k_3 = 13$; e, $k_3 = 20$; f, $k_3 = 40$.

at a rate

$$V_{3'} = k'_3\,[\text{Fru-1,6-}P_2]^{\alpha'}, \qquad [11]$$

a new activator, denoted by $X$, for the PKase enzyme. Thus, Fru-1,6-$P_2$ is no longer an effector in the PKase reaction, and a linear stability analysis of that reaction now has to involve the metabolite $X$ in conjunction with $P$-$e$Prv. Results for the ratio $T/T_{\text{PKase}}$ in that modified reaction scheme are shown in Fig. 3. The values of the parameters were chosen in the range of those investigated in Fig. 1, except for the presence here of the two additional parameters $k'_3$ and $\alpha'$. The $T$ curves are essentially insensitive to $T_{\text{PKase}}$; they show almost no dependence on the distance from marginal stability and their behavior is similar to that in the unmodified scheme far away from resonance (see Fig. 1, curve a). The addition of a reverse reaction for step 3′ did not modify the results.

To conclude, it is the coupling of the PFKase and of the PKase reactions via the Fru-1,6-$P_2$ intermediate that may lead to resonance tuning of the PFKase reaction by the PKase reaction in a restricted range of kinetic parameters. The best experimental values lie within that range. Decoupling has been done in all previous models (5), and it leads to destruction of these features.

## APPENDIX

The partial derivatives of the flows are given below.

$$\frac{\partial V_2}{\partial[\text{ATP}]} = -V_2^m\,[\text{Fru-6-}P]^n\,K_2R_2[\text{ATP}]^{n-1}n \left([\text{AMP}] - [\text{ATP}]\frac{\partial[\text{AMP}]}{\partial[\text{ATP}]}\right)\Big/([\text{AMP}]^{n+1}D_2) \qquad [A1]$$

$$\frac{\partial V_4}{\partial[\text{ATP}]} = -V_4^m\,[P\text{-}e\text{Prv}]^\gamma K_4R_4[\text{ATP}]^{m-1}m/([\text{Fru-1,6-}P_2]^mD_4) \qquad [A2]$$

$$\frac{\partial V_6}{\partial[\text{ATP}]} = k_6 \qquad [A3]$$

$$\frac{\partial V_3}{\partial[\text{Fru-1,6-}P_2]} = \alpha k_3[\text{Fru-1,6-}P_2]^{\alpha-1} \qquad [A4]$$

$$\frac{\partial V_4}{\partial[\text{Fru-1,6-}P_2]} = V_4^m[P\text{-}e\text{Prv}]^\gamma K_4R_4[\text{ATP}]^m m/([\text{Fru-1,6-}P_2]^{m+1}D_4) \qquad [A5]$$

$$\frac{\partial V_3}{\partial[P\text{-}e\text{Prv}]} = -\beta\bar{k}_3[P\text{-}e\text{Prv}]^{\beta-1} \qquad [A6]$$

$$\frac{\partial V_4}{\partial[P\text{-}e\text{Prv}]} = V_4^m\gamma[P\text{-}e\text{Prv}]^{\gamma-1}K_4\left(1 + R_4\frac{[\text{ATP}]^m}{[\text{Fru-1,6-}P_2]^m}\right)\Big/D_4, \qquad [A7]$$

where

$$D_2 = \left(K_2 + K_2R_2\frac{[\text{ATP}]^n}{[\text{AMP}]^n} + [\text{Fru-6-}P]^n\right)^2 \qquad [A8]$$

$$D_4 = \left(K_4 + K_4R_4\frac{[\text{ATP}]^m}{[\text{Fru-1,6-}P_2]^m} + [P\text{-}e\text{Prv}]^\gamma\right)^2, \qquad [A9]$$

and

$$\frac{\partial[\text{AMP}]}{\partial[\text{ATP}]} = -1 - \frac{\partial[\text{ADP}]}{\partial[\text{ATP}]} \qquad [A10]$$

$$\frac{\partial[\text{ADP}]}{\partial[\text{ATP}]} = (-1 + \{[\text{ATP}] + 2K(\text{A(MDT)P} - 2[\text{ATP}])\}/\{[\text{ATP}]^2 + 4K[\text{ATP}](\text{A(MDT)P} - [\text{ATP}])\}^{1/2})/2K. \qquad [A11]$$

We thank Dr. Peter H. Richter for helpful discussions. This work was supported in part by the U.S. Department of Energy. Y.T. acknowledges a fellowship from the North Atlantic Treaty Organization.

1. Termonia, Y. & Ross, J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2952–2956.
2. Richter, P. H. & Ross, J. (1978) *J. Chem. Phys.* **69**, 5521–5531.
3. Richter, P. H. & Ross, J. (1980) *Biophys. Chem.* **12**, 285–297.
4. Termonia, Y. & Ross, J. (1981) *J. Chem. Phys.* **74**, 2339–2345.
5. Hess, B. & Plesser, T. (1979) *Ann. N.Y. Acad. Sci.* **316**, 203–213.
6. Hess, B., Boiteux, A. & Krüger, J. (1969) *Adv. Enzyme Regul.* **7**, 149–167.
7. Dynnik, V. V. & Selkov, E. E. (1973) *FEBS Lett.* **37**, 342–346.