Biochemistry (Moscow), Vol. 69, No. 12, 2004, pp. 1324-1335. Translated from Biokhimiya, Vol. 69, No. 12, 2004, pp. 1625-1638.

Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM04-075, August 29, 2004.

Kinetic Model of Imidazologlycerol-Phosphate Synthetase from *Escherichia coli*

O. V. Demin[1]*, I. I. Goryanin[2], S. Dronov[2], and G. V. Lebedeva[1]

[1]Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, 119992 Moscow, Russia; fax: (7-095) 939-3181; E-mail: demin@genebee.msu.su
[2]GlaxoSmithKline, Gunnels Wood Road, Stevenage SG1 2NY, UK; E-mail: iig2468@gsk.com

Received March 18, 2004
Revision received June 4, 2004

Abstract—Based on the available experimental data, we developed a kinetic model of the catalytic cycle of imidazologlycerol-phosphate synthetase from *Escherichia coli* accounting for the synthetase and glutaminase activities of the enzyme. The rate equations describing synthetase and glutaminase activities of imidazologlycerol-phosphate synthetase were derived from this catalytic cycle. Using the literature data, we evaluated all kinetic parameters of the rate equations characterizing individually synthetase and glutaminase activities as well as the contribution of each activity depending on concentration of the substrates, products, and effectors. As shown, in the presence of 5′-phosphoribosylformimino-5-aminoimidazolo-4-carboxamideribonucleotide (ProFAR) and imidazologlycerol phosphate (IGP) glutaminase activity dominates over synthetase activity at sufficiently low concentrations of 5′-phosphoribulosylformimino-5-aminoimidazolo-4-carboxamideribonucleotide (PRFAR). Increased PRFAR concentrations resulted in decreased contribution of glutaminase activity and, consequently, increased the contribution of synthetase activity in the enzyme functioning.

Key words: imidazologlycerol-phosphate synthetase, catalytic cycle, model

Imidazologlycerol-phosphate synthetase (IGPS) belongs to the group of aminotransferases that catalyze the transfer of the amido group of glutamine onto various acceptor molecules—intermediates in biosynthesis of purine and pyrimidine nucleotides and amino acids [1]. Imidazologlycerol-phosphate synthetase from *Escherichia coli* is a key enzyme in biosynthesis of histidine and structurally is a heterodimer encoded by *hisH* and *hisF* [2]. Similar to other glutamine aminotransferases, IGPS catalyzes two reactions [1, 2]:

$$\text{PRFAR} + \text{Gln} = \text{AICAR} + \text{IGP} + \text{Glu}, \quad (1)$$

$$\text{Gln} = \text{Glu} + \text{NH}_3. \quad (2)$$

The essence of the first reaction is transfer of the amido group of glutamine conjugated with decomposition of 5′-phosphoribulosylformimino-5-aminoimidazolo-4-carboxamideribonucleotide (PRFAR) into 5-aminoimidazolo-4-carboxamido-1-β-D-ribofuranosyl 5′-monophosphate (AICAR) and imidazologlycerol phosphate (IGP) and synthesis of the imidazole ring. This reaction is catalyzed due to the synthetase activity of IGPS. In the second reaction, glutamine (Gln) is decomposed to glutamate (Glu). The rate of this reaction is governed by the glutaminase activity of IGPS.

In the present work, we developed a kinetic model of the catalytic cycle of imidazologlycerol-phosphate synthetase, derived the rate equations for glutaminase and synthetase activities of the enzyme, and evaluated the unknown parameters characterizing kinetic properties of this enzyme. Based on the rate equations and parameter values obtained, we studied how concentrations of the substrates and effectors determine the contributions of glutaminase and synthetase activities into the enzyme functioning.

METHODS OF INVESTIGATION

Experimental data. In this study, we used the following available data on structural and functional properties of IGPS.

1. The enzyme has two catalytic sites: one for binding to glutamine and another to PRFAR [1, 2].

* To whom correspondence should be addressed.

2. Binding to the substrates and dissociation of the products occur accidentally [1, 2].

3. The amido group cleaved from glutamine is transferred on the catalytic site binding PRFAR without emerging into solution, that is, an intramolecular transfer of the amido group occurs [1, 2].

4. The glutaminase activity of imidazologlycerol-phosphate synthetase manifests itself essentially only with excess glutamine and depletion of PRFAR, the second substrate [3, 4].

5. Whereas IGP, one of the products of the synthetase reaction catalyzed by imidazologlycerol-phosphate synthetase, catalyzes the glutaminase reaction, another product, AICAR, does not effect the latter [3, 4].

6. On the pathway of biosynthesis of histidine, the reaction catalyzed by IGPS is preceded by another reaction catalyzed by ProFAR isomerase, its substrate, 5′-phosphoribosylformimino-5-aminoimidazolo-4-carboxamidoribonucleotide (ProFAR), being an activator of the glutaminase activity of IGPS [3, 4].

Some kinetic properties of the enzyme functioning as synthetase, i.e., catalyzing only reaction (1), were investigated earlier [3]. The authors determined (at pH 8 and 25°C) Michaelis constants for Gln and PRFAR:

$$K^{s}_{m,Gln} = 240\ \mu M,\ K^{s}_{m,PRFAR} = 1.5\ \mu M \qquad (3)$$

and the maximal number of cycles of the enzyme:

$$k^{s}_{cat} = 8.5\ sec^{-1}. \qquad (4)$$

In the same work Klem and Davisson evaluated the Michaelis constant for Gln and the maximal number of cycles of the enzyme functioning as glutaminase, i.e., catalyzing only reaction (2):

$$K^{g}_{m,Gln} = 4800\ \mu M,\ k^{g}_{cat} = 0.07\ sec^{-1}. \qquad (5)$$

To characterize quantitatively the effect of ProFAR and IGP on the glutaminase activity of IGPS, Klem and Davisson evaluated the apparent Michaelis constant for Gln and apparent maximal number of cycles of the enzyme functioning as glutaminase in the presence of the activators ProFAR and IGP:

$$[ProFAR]_0 = 2000\ \mu M,$$

$$K^{g,ProFAR}_{m,app,Gln} = 2800\ \mu M,$$

$$k^{g,ProFAR}_{cat,app} = 2.6\ sec^{-1}, \qquad (6)$$

$$[IGP]_0 = 9000\ \mu M,$$

$$K^{g,IGP}_{m,app,Gln} = 1900\ \mu M,$$

$$k^{g,IGP}_{cat,app} = 2.7\ sec^{-1}. \qquad (7)$$

Kinetic characteristics of the enzyme functioning, that is, changes in concentrations of the substrates and products in the presence of 9 nM IGPS with time, were also monitored in [3].

Catalytic cycle. The experimental data mentioned in items 1-6 of the previous section were accounted for on development of kinetic model of the catalytic cycle of imidazologlycerol-phosphate synthetase presented in Fig. 1. We suggested that the enzyme functions via the Random Bi Ter mechanism according to the Cleland classification [5]; this agrees with items 1 and 2 of the previous section. Transfer of the triple enzyme–substrate complex Gln·E·PRFAR into the enzyme–product complex Glu·E·IGP·AICAR corresponds with an intermolecular transfer of the amido group mentioned in item 3 of the previous section and subsequent transformation of PRFAR into AICAR and IGP. As a result of accidental order of the substrate binding, in the absence of PRFAR a complex of the enzyme with glutamine Gln·E is formed and glutaminase reaction (2) catalyzed by free enzyme proceeds (E → Gln·E → Glu·E* → E); this corresponds with item 4 of the previous section. Activation of the glutaminase reaction in the presence of ProFAR and IGP described in items 5 and 6 of the previous section is accounted for due to inclusion of the additional reaction cycles (E → E·ProFAR → Gln·E·ProFAR → Glu·E*·ProFAR → E·ProFAR) and (E·IGP → Gln·E·IGP → Glu·E*·IGP → E·IGP) into the standard Random Bi Ter mechanism. In the present work, we did not study how the rate of glutaminase reaction depends on concentration of one of its products (NH_3); that is why NH_3 is not presented directly in Fig. 1.

Deduction of the rate equations. According to the scheme of the catalytic cycle of IGPS presented in Fig. 1, the rate equations for synthetase and glutaminase reactions can be written as follows:

$$v_s = k_1[Gln{\cdot}E{\cdot}PRFAR] - k_{-1}[Glu{\cdot}E{\cdot}IGP{\cdot}AICAR], \quad (8)$$

$$v_g = (k_2[Gln{\cdot}E] - k_{-2}[Glu{\cdot}E^*]) +$$

$$(k_3[Gln{\cdot}E{\cdot}IGP] - k_{-3}[Glu{\cdot}E^*{\cdot}IGP]) +$$

$$(k_4[Gln{\cdot}E{\cdot}ProFAR] - k_{-4}[Glu{\cdot}E^*{\cdot}ProFAR]). \quad (9)$$

Deducing the equations which describe the dependence of the rates of synthetase and glutaminase reactions on concentrations of the substrates, products, and effectors, we suggested that the rates of all reactions of the substrate binding and dissociation of the products are significantly higher than the rates of catalytic reactions designated as *1*, *2*, *3*, and *4* in Fig. 1. This suggestion allowed obtaining analytical expressions for concentrations of the enzyme states being the terms of Eqs. (8) and (9) (Gln·E·PRFAR, Glu·E·IGP·AICAR, Gln·E, Glu·E*,

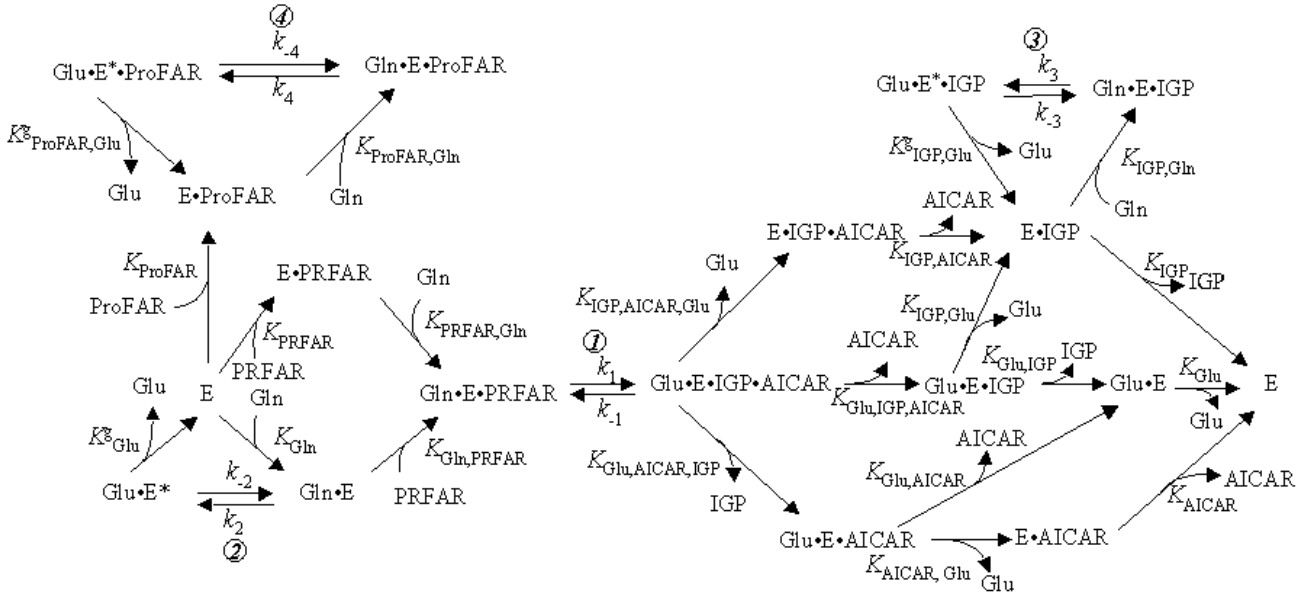

Fig. 1. Catalytic cycle of imidazologlycerol-phosphate synthetase. The dissociation constants of the substrate–product complexes and the rate constants are presented near the corresponding stages of the catalytic cycle.

Gln·E·IGP, Glu·E*·IGP, Gln·E·ProFAR, and Glu·E*·ProFAR), and thus, deduction of dependence of the rates of synthetase and glutaminase reactions on concentrations of the substrates, products, and effectors (Appendix 1):

$$v_s = \frac{[\mathrm{HisHF}]}{\Delta}\left(k_1\frac{[\mathrm{Gln}]}{K_{\mathrm{PRFAR,Gln}}}\frac{[\mathrm{PRFAR}]}{K_{\mathrm{PRFAR}}} - k_{-1}\frac{[\mathrm{AICAR}]}{K_{\mathrm{Glu,IGP,AICAR}}}\frac{[\mathrm{IGP}]}{K_{\mathrm{Glu,IGP}}}\frac{[\mathrm{Glu}]}{K_{\mathrm{Glu}}}\right);$$

$$v_g = \frac{[\mathrm{HisHF}]}{\Delta}\left(\frac{k_2}{K_{\mathrm{Gln}}} + \frac{k_3}{K_{\mathrm{IGP,Gln}}}\frac{[\mathrm{IGP}]}{K_{\mathrm{IGP}}} + \frac{k_4}{K_{\mathrm{ProFAR,Gln}}}\frac{[\mathrm{ProFAR}]}{K_{\mathrm{ProFAR}}}\right)\left([\mathrm{Gln}] - \frac{k_{-2}}{k_2}\frac{K_{\mathrm{Gln}}}{K^{\mathrm{g}}_{\mathrm{Glu}}}[\mathrm{Glu}]\right);$$

$$\Delta = 1 + \frac{[\mathrm{PRFAR}]}{K_{\mathrm{PRFAR}}} + \frac{[\mathrm{Gln}]}{K_{\mathrm{PRFAR,Gln}}}\frac{[\mathrm{PRFAR}]}{K_{\mathrm{PRFAR}}} + \frac{[\mathrm{Gln}]}{K_{\mathrm{Gln}}} + \frac{[\mathrm{Glu}]}{K^{\mathrm{g}}_{\mathrm{Glu}}} + \frac{[\mathrm{ProFAR}]}{K_{\mathrm{ProFAR}}} \cdot$$
$$\cdot\left(1 + \frac{[\mathrm{Glu}]}{K^{\mathrm{g}}_{\mathrm{ProFAR,Glu}}} + \frac{[\mathrm{Gln}]}{K_{\mathrm{ProFAR,Gln}}}\right) + \frac{[\mathrm{IGP}]}{K_{\mathrm{IGP}}}\left(1 + \frac{[\mathrm{Glu}]}{K^{\mathrm{g}}_{\mathrm{IGP,Glu}}} + \frac{[\mathrm{Gln}]}{K_{\mathrm{IGP,Gln}}}\right) + \frac{[\mathrm{AICAR}]}{K_{\mathrm{AICAR}}} +$$
$$+ \frac{[\mathrm{Glu}]}{K_{\mathrm{AICAR,Glu}}}\frac{[\mathrm{AICAR}]}{K_{\mathrm{AICAR}}} + \frac{[\mathrm{Glu}]}{K_{\mathrm{Glu}}} + \frac{[\mathrm{IGP}]}{K_{\mathrm{Glu,IGP}}}\frac{[\mathrm{Glu}]}{K_{\mathrm{Glu}}} + \frac{[\mathrm{AICAR}]}{K_{\mathrm{IGP,AICAR}}}\frac{[\mathrm{IGP}]}{K_{\mathrm{IGP}}} +$$
$$+ \frac{[\mathrm{AICAR}]}{K_{\mathrm{Glu,IGP,AICAR}}}\frac{[\mathrm{IGP}]}{K_{\mathrm{Glu,IGP}}}\frac{[\mathrm{Glu}]}{K_{\mathrm{Glu}}}. \qquad (10)$$

Here [HisHF] is the total concentration of imidazologlycerol-phosphate synthetase; K_A and K^g_A are the dissociation constants of the substrate (or product) A from free enzyme; K_{BA} and K^g_{BA} are the dissociation constants of the substrate (or product) A from the enzyme complex with the substrate (or product) B; K_{CBA} are the dissociation constants of the substrate (or product) A from the enzyme complexes with the substrates (or products) B and C; k_i, k_{-i} ($i = 1, 2, 3, 4$) are the rate constants of catalytic stages of the enzyme cycle. The dissociation and rate constants are presented in Fig. 1 near the corresponding reactions.

In Eqs. (10), there are 22 parameters—the rate and dissociation constants characterizing kinetic properties of certain stages of the catalytic cycle presented in Fig. 1. However, in the enzymatic kinetics the rate equations are usually written using parameters, which characterize kinetic properties of the enzyme as a whole. The Michaelis constants, the maximal number of the enzyme cycles, and the equilibrium constant are used as such kinetic parameters [6]. To describe kinetic properties of imidazologlycerol-phosphate synthetase, we used 17 kinetic parameters of this kind. The synthetase reaction was characterized by the maximal number of cycles of the enzyme catalyzing this reaction (k^s_{cat}), the equilibrium constant of synthetase reaction (K^s_{eq}), and the Michaelis constants of the substrates and products of this reaction ($K^s_{m,PRFAR}$, $K^s_{m,Gln}$, $K^s_{m,Glu}$, $K^s_{m,IGP}$, and $K^s_{m,AICAR}$). The glutaminase reaction in the absence of activators was characterized by the maximal number of cycles of the enzyme catalyzing this reaction (k^g_{cat}), the equilibrium constant of glutaminase reaction (K^g_{eq}), and the Michaelis constants of the substrates and products of this reaction ($K^g_{m,Gln}$ and $K^g_{m,Glu}$). To account for the effect of activators on the glutaminase reaction, we entered the following parameters: the maximal number of the enzyme cycles at infinitely high concentration of IGP ($k^{g,IGP}_{cat}$) or ProFAR ($k^{g,ProFAR}_{cat}$) and the Michaelis constants of the substrates and products of the glutaminase reaction at infinitely high concentration of IGP ($K^{g,IGP}_{m,Gln}$ and $K^{g,IGP}_{m,Glu}$) or ProFAR ($K^{g,ProFAR}_{m,Gln}$ and $K^{g,ProFAR}_{m,Glu}$). We found functional interrelations

between thus defined kinetic parameters of the imidazologlycerol-phosphate synthetase and parameters characterizing kinetic properties of certain stages of its catalytic cycle. Using this interrelation, we expressed parameters of the catalytic cycle via kinetic parameters (see Appendix 2) and wrote the rate equations (10) using traditional kinetic parameters:

$$v_s = \frac{[\text{HisHF}]}{\Delta}\frac{k^s_{cat}}{K^s_{m,\text{PRFAR}}K^g_{m,\text{Gln}}}\left([\text{PRFAR}][\text{Gln}] - [\text{AICAR}][\text{IGP}][\text{Glu}]/K^s_{eq}\right);$$

$$v_g = \frac{[\text{HisHF}]}{\Delta}\left(\frac{k^g_{cat}}{K^g_{m,\text{Gln}}} + \frac{k^{g,\text{IGP}}_{cat}}{K^{g,\text{IGP}}_{m,\text{Gln}}}\frac{[\text{IGP}]}{K_{\text{IGP}}} + \frac{k^{g,\text{ProFAR}}_{cat}}{K^{g,\text{ProFAR}}_{m,\text{Gln}}}\frac{[\text{ProFAR}]}{K_{\text{ProFAR}}}\right)\left([\text{Gln}] - [\text{Glu}]/K^g_{eq}\right);$$

$$\Delta = 1 + \frac{[\text{Gln}]}{K^g_{m,\text{Gln}}} + \frac{[\text{PRFAR}]}{K^s_{m,\text{PRFAR}}}\left(\frac{K^s_{m,\text{Gln}}}{K^g_{m,\text{Gln}}} + \frac{[\text{Gln}]}{K^g_{m,\text{Gln}}}\right) + \frac{[\text{ProFAR}]}{K_{\text{ProFAR}}}\left(1 + \frac{[\text{Glu}]}{K^{g,\text{ProFAR}}_{m,\text{Glu}}} + \frac{[\text{Gln}]}{K^{g,\text{ProFAR}}_{m,\text{Gln}}}\right) +$$
$$+ \frac{[\text{IGP}]}{K_{\text{IGP}}}\left(1 + \frac{[\text{Glu}]}{K^{g,\text{IGP}}_{m,\text{Glu}}} + \frac{[\text{Gln}]}{K^{g,\text{IGP}}_{m,\text{Gln}}}\right) + \frac{[\text{Glu}]}{K^g_{m,\text{Glu}}} + \frac{[\text{AICAR}]}{K_{\text{AICAR}}} + \frac{[\text{Glu}]}{K_{\text{Glu}}}\frac{[\text{IGP}]}{K_{\text{Glu,IGP}}} + \left(1 + \frac{K_{\text{IGP}}K^{g,\text{IGP}}_{m,\text{Glu}}}{K_{\text{Glu,IGP}}K_{\text{Glu}}}\right)\cdot$$
$$\cdot\left(\frac{[\text{AICAR}]}{K^s_{m,\text{AICAR}}}\frac{[\text{Glu}]}{K^{g,\text{IGP}}_{m,\text{Glu}}}\frac{K^s_{m,\text{IGP}}}{K_{\text{IGP}}} + \frac{[\text{AICAR}]}{K^s_{m,\text{AICAR}}}\frac{[\text{IGP}]}{K_{\text{IGP}}}\frac{K^s_{m,\text{Glu}}}{K^{g,\text{IGP}}_{m,\text{Glu}}} + \frac{[\text{AICAR}]}{K^s_{m,\text{AICAR}}}\frac{[\text{IGP}]}{K_{\text{IGP}}}\frac{[\text{Glu}]}{K^{g,\text{IGP}}_{m,\text{Glu}}}\right). \quad (11)$$

In Eqs. (11), there are 22 parameters: 17 are kinetic parameters and the other five are the dissociation constants of the products and effectors (K_{AICAR}, K_{Glu}, K_{IGP}, $K_{\text{Glu,IGP}}$, and K_{ProFAR}); as shown in Appendix 2, the dissociation constant of glutamate from the free enzyme must be higher than the Michaelis constant for glutamate in the glutaminase reaction:

$$K^g_{m,\text{Glu}} < K_{\text{Glu}}. \quad (12)$$

Methods for study of IGPS models. All the models presented in this work were studied using the DBSolve 5.0 program package [7]. We obtained time-dependent variables in the given system of differential equations with the given initial conditions by the methods of numerical integration supplied by this package. The Hook–Jeaves algorithm was used to identify parameter values from experimental data.

RESULTS AND DISCUSSION

Evaluation of parameters of the rate equations. To find the values of parameters, which appear in Eqs. (11), we used experimental data from [3, 4]. As mentioned in "Methods of Investigation", these authors evaluated Michaelis constants for Gln and PRFAR (3) and the maximal number of cycles (4) of the enzyme catalyzing only the synthetase reaction (1). The Michaelis constant for Gln and the maximal number of cycles (5) of the enzyme functioning as glutaminase, that is, catalyzing only reaction (2) were also evaluated in [3, 4]. Besides this, Klem and Davisson [3] found the values of apparent Michaelis constants for glutamine and apparent maximal number of cycles of the enzyme catalyzing only the glutaminase reaction in the presence of 2 mM ProFAR (6) or 9 mM IGP (7). We evaluated five kinetic parameters appearing in Eqs. (11) using Eqs. (3)-(5). The values of the apparent constants from (6) and (7) were used to obtain four relationships between the remaining 17 parameters; this allowed reducing the number of unknown parameters to 13. These four relationships are algebraic expressions for the apparent constants, whose values are given in (6) and (7) via the parameters of Eqs. (11).

To derive expressions for the apparent Michaelis constant for glutamine and the apparent maximal number of cycles of the enzyme catalyzing only the glutaminase reaction in the presence of 2 mM ProFAR, we rewrote the rate equation of the glutaminase reaction accounting for conditions of the experiment, which gave evaluation of these apparent constants. In fact, in this experiment we measured the initial rate of imidazologlycerol-phosphate synthetase depending on the glutamine concentration at various concentrations of the substrates, products, and effectors: [PRFAR] = [Glu] = [IGP] = [AICAR] = 0, [ProFAR] = $[\text{ProFAR}]_0$ = 2 mM. Substitution of these concentration values into the rate equation for glutaminase reaction (11) gives:

$$v_g = \frac{[\text{HisHF}]\left(\frac{k^g_{cat}}{K^g_{m,\text{Gln}}} + \frac{k^{g,\text{ProFAR}}_{cat}}{K^{g,\text{ProFAR}}_{m,\text{Gln}}}\frac{[\text{ProFAR}]_0}{K_{\text{ProFAR}}}\right)[\text{Gln}]}{1 + \frac{[\text{Gln}]}{K^g_{m,\text{Gln}}} + \frac{[\text{ProFAR}]_0}{K_{\text{ProFAR}}}\left(1 + \frac{[\text{Gln}]}{K^{g,\text{ProFAR}}_{m,\text{Gln}}}\right)}. \quad (13)$$

From Eq. (13), we obtained the following expressions for the apparent Michaelis constant for glutamine and apparent maximal number of cycles of the enzyme:

$$K^{g,\text{ProFAR}}_{m,\text{app,Gln}} = \frac{1 + \frac{[\text{ProFAR}]_0}{K_{\text{ProFAR}}}}{\frac{1}{K^g_{m,\text{Gln}}} + \frac{1}{K^{g,\text{ProFAR}}_{m,\text{Gln}}}\frac{[\text{ProFAR}]_0}{K_{\text{ProFAR}}}},$$

$$k^{g,\text{ProFAR}}_{cat,\text{app}} = \frac{\frac{k^g_{cat}}{K^g_{m,\text{Gln}}} + \frac{k^{g,\text{ProFAR}}_{cat}}{K^{g,\text{ProFAR}}_{m,\text{Gln}}}\frac{[\text{ProFAR}]_0}{K_{\text{ProFAR}}}}{\frac{1}{K^g_{m,\text{Gln}}} + \frac{1}{K^{g,\text{ProFAR}}_{m,\text{Gln}}}\frac{[\text{ProFAR}]_0}{K_{\text{ProFAR}}}}. \quad (14)$$

These two relationships allowed expressing K_{ProFAR} and $k^{g,\text{ProFAR}}_{cat}$ as functions of the other kinetic parameters of Eqs. (11), the apparent Michaelis constant, the apparent catalytic constant, and $[\text{ProFAR}]_0$ value at which these constants were measured:

$$K_{\text{ProFAR}} = [\text{ProFAR}]_0 \frac{K^{\text{g}}_{\text{m,Gln}}\left(K^{\text{g,ProFAR}}_{\text{m,app,Gln}} - K^{\text{g,ProFAR}}_{\text{m,Gln}}\right)}{K^{\text{g,ProFAR}}_{\text{m,Gln}}\left(K^{\text{g}}_{\text{m,Gln}} - K^{\text{g,ProFAR}}_{\text{m,app,Gln}}\right)}; \quad (15)$$

$$k^{\text{g,ProFAR}}_{\text{cat}} = k^{\text{g,ProFAR}}_{\text{cat,app}} + \left(k^{\text{g,ProFAR}}_{\text{cat,app}} - k^{\text{g}}_{\text{cat}}\right)\frac{K^{\text{g,ProFAR}}_{\text{m,app,Gln}} - K^{\text{g,ProFAR}}_{\text{m,Gln}}}{K^{\text{g}}_{\text{m,Gln}} - K^{\text{g,ProFAR}}_{\text{m,app,Gln}}}. \quad (16)$$

Since K_{ProFAR} and $k^{\text{g,ProFAR}}_{\text{cat}}$ are to be always positive, the values of parameters of the right parts of Eqs. (15) and (16) should satisfy the following inequalities:

$$K^{\text{g}}_{\text{m,Gln}} > K^{\text{g,ProFAR}}_{\text{m,app,Gln}} > K^{\text{g,ProFAR}}_{\text{m,Gln}},$$

$$k^{\text{g,ProFAR}}_{\text{cat,app}} > k^{\text{g}}_{\text{cat}}. \quad (17)$$

Using the data on the apparent kinetic parameters of the glutaminase reaction activated by IGP (7) and repeating all the reasons and manipulations presented above, we obtained relationships which allowed us expressing K_{IGP} and $k^{\text{g,IGP}}_{\text{cat}}$ as functions of other kinetic parameters of Eqs. (11), the apparent Michaelis constant, the apparent catalytic constant, and $[\text{IGP}]_0$ value at which these constants were measured:

$$K_{\text{IGP}} = [\text{IGP}]_0 \frac{K^{\text{g}}_{\text{m,Gln}}\left(K^{\text{g,IGP}}_{\text{m,app,Gln}} - K^{\text{g,IGP}}_{\text{m,Gln}}\right)}{K^{\text{g,IGP}}_{\text{m,Gln}}\left(K^{\text{g}}_{\text{m,Gln}} - K^{\text{g,IGP}}_{\text{m,app,Gln}}\right)},$$

$$k^{\text{g,IGP}}_{\text{cat}} = k^{\text{g,IGP}}_{\text{cat,app}} + \left(k^{\text{g,IGP}}_{\text{cat,app}} - k^{\text{g}}_{\text{cat}}\right)\frac{K^{\text{g,IGP}}_{\text{m,app,Gln}} - K^{\text{g,IGP}}_{\text{m,Gln}}}{K^{\text{g}}_{\text{m,Gln}} - K^{\text{g,IGP}}_{\text{m,app,Gln}}}; \quad (18)$$

$$K^{\text{g}}_{\text{m,Gln}} > K^{\text{g,IGP}}_{\text{m,app,Gln}} > K^{\text{g,IGP}}_{\text{m,Gln}},$$

$$k^{\text{g,IGP}}_{\text{cat,app}} > k^{\text{g}}_{\text{cat}}. \quad (19)$$

Thus, accounting for the values of kinetic parameters (3)-(5) and using Eqs. (15), (16), and (18), we reduced the number of unknown parameters involved in the rate equation for IGPS from 22 to 13, and these 13 parameters should satisfy inequalities (12), (17), and (19).

To obtain the values of the remaining 13 parameters, we used kinetic data for imidazologlycerol-phosphate synthetase obtained at pH 8.0 and 25°C [3]. Experimental conditions were as follows: to 90 μM PRFAR and 5 mM glutamine imidazologlycerol-phosphate synthetase was added to the final concentration 9 nM, and changes in PRFAR, AICAR, and Glu concentrations with time were monitored. Kinetic model describing this experiment can be depicted as the kinetic scheme presented in Fig. 2; this model is given by a system of differential and algebraic equations:

$$\begin{cases} d[\text{PRFAR}]/dt = -v_{\text{s}}, \\ d[\text{Glu}]/dt = v_{\text{s}} + v_{\text{g}}, \\ [\text{Gln}] + [\text{Glu}] = 5000\ \mu\text{M}, \\ [\text{PRFAR}] + [\text{AICAR}] = 90\ \mu\text{M}, \\ [\text{PRFAR}] + [\text{IGP}] = 90\ \mu\text{M}. \end{cases} \quad (20)$$

The initial values of variables in this model coincide with the initial experimental concentrations: [PRFAR] = 90 μM, [Gln] = 5000 μM, [Glu] = [IGP] = [AICAR] = 0.

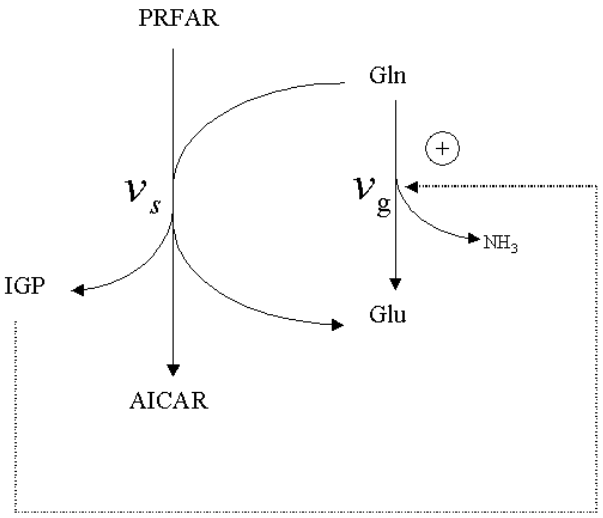

Fig. 2. Kinetic scheme of the model corresponding with the experimental data on the kinetics of imidazologlycerol-phosphate synthetase [3]. Solid arrows specify synthetase (v_s) and glutaminase (v_g) activities of the enzyme, and broken arrow shows that IGP activates the glutaminase reaction.

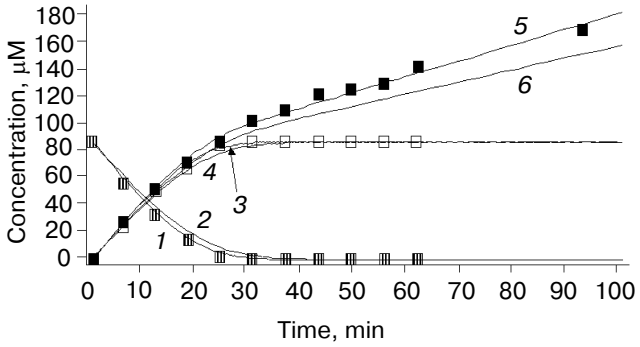

Fig. 3. Time dependences of PRFAR (curves *1* and *2* and hatched squares), AICAR (curves *3* and *4* and open squares), and Glu (curves *5* and *6* and black squares) concentrations, experimentally measured in [3] (hatched, open, and black squares) and obtained from the kinetic model based on this experiment (curves *1*-*6*).

Eleven of 13 kinetic parameters of the rate equations of the synthetase and glutaminase reactions were fitted so that time dependences of PRFAR, AICAR, and Glu concentrations obtained as numerical solution of system (20) best coincided with corresponding experimental dependences [3]. The values of the two parameters $K^{g,ProFAR}_{m,Gln}$ and $K^{g,ProFAR}_{m,Glu}$ characterizing kinetic properties of the enzyme in the presence of ProFAR (activator of the glutaminase reaction) could not be evaluated from the experimental data [3] because of zero concentration of this activator in the considered experiment. Figure 3 presents experimental data [3] (squares) and model results (solid lines). Approximation of the experimental data (Fig. 3, curves *2*, *4*, and *6*) appeared to be impossible with sufficient range of accuracy by changing values of 11 parameters, which remained non-evaluated (that is, free) after accounting for the experimental data [3, 4] by the values of true and apparent catalytic and Michaelis constants in our model. However, if we suppose that the values of these true and apparent catalytic and Michaelis constants from expressions (3)-(7) are measured with 30% accuracy and that is why we can vary these values within this 30% interval together with 11 non-evaluated parameters, the results of modeling will exactly coincide with experimental data (Fig. 3, curves *1*, *3*, and 5). The thus fitted parameter values are given in the table.

The remaining two parameters $K^{g,ProFAR}_{m,Gln}$ and $K^{g,ProFAR}_{m,Glu}$ characterizing kinetic properties of imidazologlycerol-phosphate synthetase in the presence of ProFAR, an activator of the glutaminase reaction, were evaluated by the data of [8]; in this work, Jurgens and coauthors studied the properties of ProFAR isomerase, a precursor of IGPS in the pathway of biosynthesis of histidine and a catalyst of the isomerization reaction of ProFAR into PRFAR. Experimental conditions were as follows: pH 7.5 and 25°C. To 0.25 μM ProFAR isomerase, 1 μM imidazologlycerol-phosphate synthetase and 5 mM glutamine ProFAR was added to the final concentration 20 μM, and the time dependence of its concentration was monitored. The kinetic model describing this experiment can be depicted as the kinetic scheme presented in Fig. 4; this model is given by a system of differential and algebraic equations:

$$\begin{cases} d[\text{ProFAR}]/dt = -v_{\text{ProFAR-isomerase}}, \\ d[\text{PRFAR}]/dt = v_{\text{ProFAR-isomerase}} - v_s^{pH}, \\ d[\text{Glu}]/dt = v_s^{pH} + v_g^{pH}, \\ [\text{Gln}] + [\text{Glu}] = 5000\ \mu\text{M}, \\ [\text{ProFAR}] + [\text{PRFAR}] + [\text{AICAR}] = 20\ \mu\text{M}, \\ [\text{ProFAR}] + [\text{PRFAR}] + [\text{IGP}] = 20\ \mu\text{M}. \end{cases} \quad (21)$$

The initial values of variables in the model coincide with the initial experimental concentrations: [ProFAR] =

Kinetic parameters of imidazologlycerol-phosphate synthetase and ProFAR isomerase

Parameter	Experimental data	Values obtained as a result of identification of the model with experimental data [3] and [8]
k^s_{cat}	510 min^{-1} [3]	585 min^{-1}
K^s_{eq}		4.85 mM
$K^s_{m,PRFAR}$	1.5 μM [3]	1.86 μM
$K^s_{m,Gln}$	240 μM [3]	180 μM
$K^s_{m,Glu}$		0.01 μM
$K^s_{m,IGP}$		0.14 μM
$K^s_{m,AICAR}$		20.6 mM
k^g_{cat}	4.2 min^{-1} [3]	5.28 min^{-1}
K^g_{eq}		1.03 mM
$K^g_{m,Gln}$	4.8 mM [3]	5.96 mM
$K^g_{m,Glu}$		51.4 mM
$k^{g,IGP}_{cat}$		210 min^{-1}
$k^{g,ProFAR}_{cat}$		156 min^{-1}
$K^{g,IGP}_{m,Gln}$		1.32 mM
$K^{g,IGP}_{m,Glu}$		10.4 mM
$K^{g,ProFAR}_{m,Gln}$		946 μM
$K^{g,ProFAR}_{m,Glu}$		46.2 mM
K_{AICAR}		31.7 mM
K_{Glu}		51.4 mM
K_{IGP}		61.3 μM
$K_{Glu,\ IGP}$		30.9 mM
K_{ProFAR}		4.7 μM
k^{HisA}_{cat}	42 min^{-1} [8]	42 min^{-1}
$K^{HisA}_{m,ProFAR}$	0.6 μM [8]	0.6 μM
$K^{HisA}_{m,PRFAR}$		0.03 μM
$k^{g,IGP}_{cat,app}$	162 min^{-1} [3]	210 min^{-1}
$K^{g,IPG}_{m,app,Gln}$	1.9 mM [3]	1.33 mM
$k^{g,ProFAR}_{cat,app}$	156 min^{-1} [3]	156 min^{-1}
$K^{g,ProFAR}_{m,app,Gln}$	2.8 mM [3]	2.8 mM
K^1_H		0.01 μM
K^2_H		0.93 μM

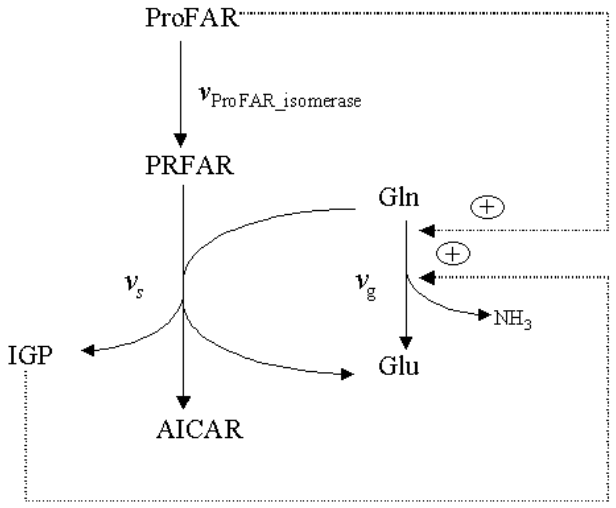

Fig. 4. Kinetic scheme of a model corresponding to the kinetic study of imidazologlycerol-phosphate synthetase–ProFAR isomerase system [8]. Solid arrows designate reactions catalyzed by ProFAR isomerase ($v_{ProFAR\_isomerase}$) and also the synthetase (v_s) and glutaminase (v_g) activities of IGPS. Broken arrows show that IGP and ProFAR stimulate the glutaminase activity of IGPS.

20 μM, [Gln] = 5000 μM, [Glu] = [PRFAR] = [IGP] = [AICAR] = 0. Since most of the imidazologlycerol-phosphate synthetase parameters were evaluated from experimental data obtained at pH 8.0 [3], for evaluation of remaining parameters from experimental data obtained at pH 7.5 [8], it is necessary to account for the pH dependence of the activity of this enzyme. Following the method for describing pH dependence of the rate of enzymatic reaction [6], we suggested that a catalytic site (one or several amino acid residues directly participating in catalysis) can be deprotonated and protonated singly or doubly, the singly protonated form of the enzyme being catalytically active. We also suggested that the values of the proton dissociation constants do not depend on the enzyme state. These suggestions do not contradict experimental data on kinetics of this enzyme; as a result, the pH dependence of the rate of IGPS functioning is given as follows:

$$v_s^{pH} = v_s \frac{1 + \frac{K_H^1}{10^{6-pH_1}} + \frac{10^{6-pH_1}}{K_H^2}}{1 + \frac{K_H^1}{10^{6-pH}} + \frac{10^{6-pH}}{K_H^2}},$$

$$v_g^{pH} = v_g \frac{1 + \frac{K_H^1}{10^{6-pH_1}} + \frac{10^{6-pH_1}}{K_H^2}}{1 + \frac{K_H^1}{10^{6-pH}} + \frac{10^{6-pH}}{K_H^2}}, \quad (22)$$

where v_s and v_g are given by Eqs. (11) describing the synthetase and glutaminase activities of imidazologlycerol-phosphate synthetase, K_H^1 and K_H^2 are the proton dissociation constants from non-protonated and singly protonated forms of the enzyme, respectively, and $pH_1 = 8.0$ coincides with pH in the experiment [3] used for evaluation of most of the enzyme constants. Since the data [8] were obtained at pH 7.5, in the rate equations for imidazologlycerol-phosphate synthetase involved in kinetic model (21), which describes these data, the pH should be also 7.5.

The rate equation for ProFAR isomerase can be written as follows:

$$v_{ProFAR\_isomerase} = \frac{k_{cat}^{HisA}[HisA][ProFAR]}{K_{m,ProFAR}^{HisA} + [ProFAR] + \frac{K_{m,ProFAR}^{HisA}}{K_{m,PRFAR}^{HisA}} \cdot [PRFAR]}. \quad (23)$$

The catalytic and Michaelis constants for ProFAR evaluated from the data obtained at pH 7.5 and 25°C were taken from [8]: $k_{cat}^{HisA} = 0.7$ sec^{-1}, $K_{m,ProFAR}^{HisA} = 0.6$ μM. The remaining unknown parameters of model (21) ($K_{m,Gln}^{g,ProFAR}$, $K_{m,Glu}^{g,ProFAR}$, $K_{m,PRFAR}^{HisA}$, K_H^1, and K_H^2) were fitted so that time dependence of ProFAR concentration obtained as a result of numerical solution of system (21) best coincided with the corresponding experimentally obtained dependence [8]. As shown in Fig. 5, the thus found parameters provide precise coincidence of the results of modeling (solid line) with experimental data [8] (squares). The values of fitted parameters are given in the table.

How the synthetase and glutaminase activities of imidazologlycerol-phosphate synthetase depend on concentrations of the substrates and effectors. As shown in [1, 2], imidazologlycerol-phosphate synthetase can catalyze two different processes: synthesis of imidazologlycerol phosphate (1) and decomposition of glutamine (2). To study what activity dominates and what is an accompanying one and how it depends on concentrations of the sub-

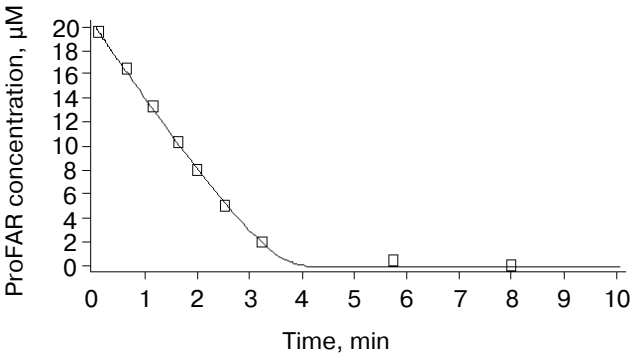

Fig. 5. Time dependence of ProFAR concentrations: experimentally measured in [8] (open squares) and obtained from the kinetic model based on this experiment (solid line).

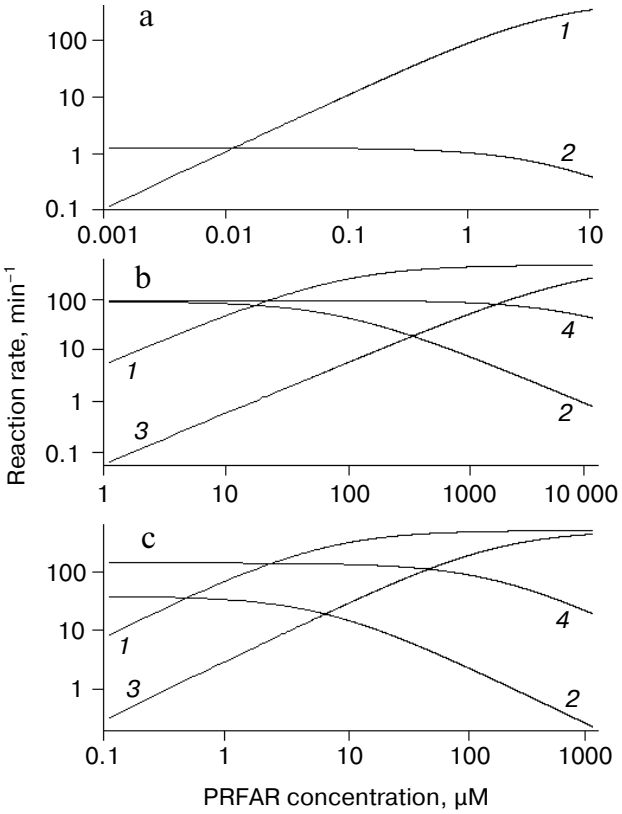

Fig. 6. Rates of the synthetase (curves *1* and *3*) and glutaminase (curves *2* and *4*) reactions catalyzed by imidazologlycerol-phosphate synthetase versus PRFAR concentration: a) in the absence of activators of the glutaminase reaction; b) at various ProFAR concentrations (10 μM ProFAR, curves *1* and *2*; 1 mM ProFAR, curves *3* and *4*); c) at various IGP concentrations (10 μM IGP, curves *1* and *2*; 1 mM IGP, curves *3* and *4*). All the curves were obtained at zero concentrations of the products and 1 mM Gln.

strates and effectors, we plotted (Fig. 6) the rates of the glutaminase and synthetase reactions versus the substrate (PRFAR) concentration at various concentrations of effectors (IGP and ProFAR), zero concentrations of the products, pH 8.0, and fixed concentration of glutamine ([Gln] = 1 mM), using the results of the previous section (the rate equations (11) and the values of parameters from the table). It appeared that in the absence of effectors the synthetase activity can be considered as dominating. In fact, as shown in Fig. 6a, at PRFAR concentrations exceeding 10 nM, the rate of the synthetase reaction is higher than the rate of the glutaminase reaction. Moreover, beginning from ~100 nM PRFAR, the rate of the synthetase reaction is more than ten times higher than that of the glutaminase reaction, and this difference grows with increase in PRFAR concentration, achieving 1000 times at [PRFAR] = 10 μM. In the presence of effector (ProFAR), the situation is not so definite. As shown in Fig. 6b, at 10 μM ProFAR the rate of the synthetase reaction (curve *1*) becomes equal to the rate of the glutaminase reaction (curve *2*) at PRFAR concentration ~13 μM. At lower PRFAR concentrations, the rate of the glutaminase reaction is higher than the rate of the synthetase reaction, whereas at higher PRFAR concentrations the synthetase activity dominates. At higher ProFAR concentrations, the interval of PRFAR concentrations corresponding to domination of the glutaminase reaction expands. In fact, at 1 mM ProFAR the rate of the synthetase reaction (curve *3*) becomes equal to the rate of the glutaminase reaction (curve *4*) at PRFAR concentration ~1300 μM. Moreover, at PRFAR concentrations lower than 130 μM the rate of the glutaminase reaction is more than ten times higher than the rate of the synthetase reaction, that is, the glutaminase activity becomes dominating. Analogous conclusions are true also for another effector—IGP (Fig. 6c). At 1 mM IGP, the rate of the synthetase reaction (curve *3*) becomes equal to the rate of the glutaminase reaction (curve *4*) at PRFAR concentration ~23 μM. As shown in Fig. 6c, increase in IGP concentration also results in expansion of the interval of PRFAR concentrations at which the glutaminase activity dominates.

As follows from Fig. 6, independent on the presence of the effectors—ProFAR and IGP—increase in the substrate (PRFAR) concentration results in decrease in glutaminase and increase in synthetase activities of imidazologlycerol-phosphate synthetase.

This work was financially supported by the Russian Foundation for Basic Research (grant No. 01-04-48242) and GlaxoSmithKline Company.

REFERENCES

1. Zalkin, H. (1993) *Adv. Enzymol. Relat. Areas Mol. Biol.*, **66**, 203-309.
2. Zalkin, H., and Smith, J. L. (1998) *Adv. Enzymol. Relat. Areas Mol. Biol.*, **72**, 87-144.
3. Klem, T. J., and Davisson, V. J. (1993) *Biochemistry*, **32**, 5177-5186.
4. Chittur, S. V., Klem, T. J., Shafer, C. M., and Davisson, V. J. (2001) *Biochemistry*, **40**, 876-887.
5. Cleland, W. W. (1963) *Biochim. Biophys. Acta*, **67**, 104-137.
6. Cornish-Bowden, A. (2001) *Fundamentals of Enzyme Kinetics*, Portland Press, USA.
7. Goryanin, I., Hodgman, T. C., and Selkov, E. (1999) *Bioinformatics*, **15**, 749-758.
8. Jurgens, C., Strom, A., Wegener, D., Hettwer, S., Wilmanns, M., and Sterner, R. (2000) *Proc. Natl. Acad. Sci. USA*, **97**, 9925-9930.

APPENDIX 1

To deduce expressions defining the rates of the synthetase and glutaminase reactions, let us suppose that all reactions of the substrate addition and dissociation of the products are much faster than the catalytic reactions designated by numbers *1*, *2*, *3*, and *4* in Fig. 1. This means that each of these "fast" reactions can be considered as a quasi-equilibrium one (the dissociation constants are given near the corresponding reactions in Fig. 1); thus, we can write the following relationships:

$$K_{\mathrm{PRFAR}} = \frac{[\mathrm{PRFAR}][\mathrm{E}]}{[\mathrm{E}\cdot\mathrm{PRFAR}]},$$

$$K_{\mathrm{PRFAR,Gln}} = \frac{[\mathrm{Gln}][\mathrm{E}\cdot\mathrm{PRFAR}]}{[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{PRFAR}]},$$

$$K_{\mathrm{Gln}} = \frac{[\mathrm{Gln}][\mathrm{E}]}{[\mathrm{Gln}\cdot\mathrm{E}]},$$

$$K^{\mathrm{g}}_{\mathrm{Glu}} = \frac{[\mathrm{Glu}][\mathrm{E}]}{[\mathrm{Glu}\cdot\mathrm{E}^*]},$$

$$K_{\mathrm{ProFAR}} = \frac{[\mathrm{ProFAR}][\mathrm{E}]}{[\mathrm{E}\cdot\mathrm{ProFAR}]},$$

$$K_{\mathrm{ProFAR,Gln}} = \frac{[\mathrm{Gln}][\mathrm{E}\cdot\mathrm{ProFAR}]}{[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{ProFAR}]},$$

$$K^{\mathrm{g}}_{\mathrm{ProFAR,Glu}} = \frac{[\mathrm{Glu}][\mathrm{E}\cdot\mathrm{ProFAR}]}{[\mathrm{Glu}\cdot\mathrm{E}^*\cdot\mathrm{ProFAR}]},$$

$$K_{\mathrm{AICAR}} = \frac{[\mathrm{AICAR}][\mathrm{E}]}{[\mathrm{E}\cdot\mathrm{AICAR}]},$$

$$K_{\mathrm{AICAR,Glu}} = \frac{[\mathrm{Glu}][\mathrm{E}\cdot\mathrm{AICAR}]}{[\mathrm{Glu}\cdot\mathrm{E}\cdot\mathrm{AICAR}]},$$

$$K_{\mathrm{Glu}} = \frac{[\mathrm{Glu}][\mathrm{E}]}{[\mathrm{Glu}\cdot\mathrm{E}]},$$

$$K_{\mathrm{Glu,IGP}} = \frac{[\mathrm{IGP}][\mathrm{Glu}\cdot\mathrm{E}]}{[\mathrm{Glu}\cdot\mathrm{E}\cdot\mathrm{IGP}]},$$

$$K_{\mathrm{Glu,IGP,AICAR}} = \frac{[\mathrm{AICAR}][\mathrm{Glu}\cdot\mathrm{E}\cdot\mathrm{IGP}]}{[\mathrm{Glu}\cdot\mathrm{E}\cdot\mathrm{IGP}\cdot\mathrm{AICAR}]},$$

$$K_{\mathrm{IGP}} = \frac{[\mathrm{IGP}][\mathrm{E}]}{[\mathrm{E}\cdot\mathrm{IGP}]},$$

$$K_{\mathrm{IGP,AICAR}} = \frac{[\mathrm{AICAR}][\mathrm{E}\cdot\mathrm{IGP}]}{[\mathrm{E}\cdot\mathrm{IGP}\cdot\mathrm{AICAR}]},$$

$$K_{\mathrm{IGP,Gln}} = \frac{[\mathrm{Gln}][\mathrm{E}\cdot\mathrm{IGP}]}{[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{IGP}]},$$

$$K^{\mathrm{g}}_{\mathrm{IGP,Glu}} = \frac{[\mathrm{Glu}][\mathrm{E}\cdot\mathrm{IGP}]}{[\mathrm{Glu}\cdot\mathrm{E}^*\cdot\mathrm{IGP}]}. \qquad \text{(A1)}$$

For concentrations of the enzyme states, the following conservation law is also fulfilled:

$$\begin{aligned}&[\mathrm{E}]+[\mathrm{E}\cdot\mathrm{PRFAR}]+[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{PRFAR}]+\\&+[\mathrm{Gln}\cdot\mathrm{E}]+[\mathrm{Glu}\cdot\mathrm{E}^*]+[\mathrm{E}\cdot\mathrm{ProFAR}]+\\&+[\mathrm{Glu}\cdot\mathrm{E}^*\cdot\mathrm{ProFAR}]+[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{ProFAR}]+\\&+[\mathrm{E}\cdot\mathrm{AICAR}]+[\mathrm{Glu}\cdot\mathrm{E}\cdot\mathrm{AICAR}]+\\&+[\mathrm{Glu}\cdot\mathrm{E}]+[\mathrm{Glu}\cdot\mathrm{E}\cdot\mathrm{IGP}]+[\mathrm{E}\cdot\mathrm{IGP}]+\\&+[\mathrm{E}\cdot\mathrm{IGP}\cdot\mathrm{AICAR}]+[\mathrm{Glu}\cdot\mathrm{E}^*\cdot\mathrm{IGP}]+\\&+[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{IGP}]+[\mathrm{Glu}\cdot\mathrm{E}\cdot\mathrm{IGP}\cdot\mathrm{AICAR}]=\\&=[\mathrm{HisHF}],\end{aligned} \qquad \text{(A2)}$$

where [HisHF] is the total concentration of imidazologlycerol-phosphate synthetase. Solving the system of linear (relative to concentrations of the enzyme states) algebraic equations (A1) and (A2), we obtain the following expressions for the enzyme forms:

$$[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{PRFAR}] = \frac{[\mathrm{Gln}]}{K_{\mathrm{PRFAR,Gln}}}\frac{[\mathrm{PRFAR}]}{K_{\mathrm{PRFAR}}}\frac{[\mathrm{HisHF}]}{\Delta},$$

$$[\mathrm{Gln}\cdot\mathrm{E}] = \frac{[\mathrm{Gln}]}{K_{\mathrm{Gln}}}\frac{[\mathrm{HisHF}]}{\Delta},$$

$$[\mathrm{Glu}\cdot\mathrm{E}^*] = \frac{[\mathrm{Glu}]}{K^{\mathrm{g}}_{\mathrm{Glu}}}\frac{[\mathrm{HisHF}]}{\Delta},$$

$$[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{IGP}] = \frac{[\mathrm{IGP}]}{K_{\mathrm{IGP}}}\frac{[\mathrm{Gln}]}{K_{\mathrm{IGP,Gln}}}\frac{[\mathrm{HisHF}]}{\Delta},$$

$$[\mathrm{Glu}\cdot\mathrm{E}^*\cdot\mathrm{IGP}] = \frac{[\mathrm{IGP}]}{K_{\mathrm{IGP}}}\frac{[\mathrm{Glu}]}{K^{\mathrm{g}}_{\mathrm{IGP,Glu}}}\frac{[\mathrm{HisHF}]}{\Delta},$$

$$[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{ProFAR}] = \frac{[\mathrm{ProFAR}]}{K_{\mathrm{ProFAR}}}\frac{[\mathrm{Gln}]}{K_{\mathrm{ProFAR,Gln}}}\frac{[\mathrm{HisHF}]}{\Delta},$$

$$[\mathrm{Glu}\cdot\mathrm{E}^*\cdot\mathrm{IGP}] = \frac{[\mathrm{ProFAR}]}{K_{\mathrm{ProFAR}}}\frac{[\mathrm{Glu}]}{K^{\mathrm{g}}_{\mathrm{ProFAR,Glu}}}\frac{[\mathrm{HisHF}]}{\Delta},$$

$$[\mathrm{Gln}\cdot\mathrm{E}\cdot\mathrm{PRFAR}] = \frac{[\mathrm{AICAR}]}{K_{\mathrm{Glu,IGP,AICAR}}}\frac{[\mathrm{IGP}]}{K_{\mathrm{Glu,IGP}}}\frac{[\mathrm{Glu}]}{K_{\mathrm{Glu}}}\frac{[\mathrm{HisHF}]}{\Delta},$$

$$\Delta = 1 + \frac{[\mathrm{PRFAR}]}{K_{\mathrm{PRFAR}}} + \frac{[\mathrm{Gln}]}{K_{\mathrm{PRFAR,Gln}}}\frac{[\mathrm{PRFAR}]}{K_{\mathrm{PRFAR}}} + \frac{[\mathrm{Gln}]}{K_{\mathrm{Gln}}} + \frac{[\mathrm{Glu}]}{K^{\mathrm{g}}_{\mathrm{Glu}}} + \frac{[\mathrm{ProFAR}]}{K_{\mathrm{ProFAR}}} \cdot$$
$$\left(1 + \frac{[\mathrm{Glu}]}{K^{\mathrm{g}}_{\mathrm{ProFAR,Glu}}} + \frac{[\mathrm{Gln}]}{K_{\mathrm{ProFAR,Gln}}}\right) + \frac{[\mathrm{IGP}]}{K_{\mathrm{IGP}}}\left(1 + \frac{[\mathrm{Glu}]}{K^{\mathrm{g}}_{\mathrm{IGP,Glu}}} + \frac{[\mathrm{Gln}]}{K_{\mathrm{IGP,Gln}}}\right) + \frac{[\mathrm{AICAR}]}{K_{\mathrm{AICAR}}} + \frac{[\mathrm{Glu}]}{K_{\mathrm{AICAR,Glu}}}\frac{[\mathrm{AICAR}]}{K_{\mathrm{AICAR}}} +$$
$$\frac{[\mathrm{Glu}]}{K_{\mathrm{Glu}}} + \frac{[\mathrm{IGP}]}{K_{\mathrm{Glu,IGP}}}\frac{[\mathrm{Glu}]}{K_{\mathrm{Glu}}} + \frac{[\mathrm{AICAR}]}{K_{\mathrm{IGP,AICAR}}}\frac{[\mathrm{IGP}]}{K_{\mathrm{IGP}}} + \frac{[\mathrm{AICAR}]}{K_{\mathrm{Glu,IGP,AICAR}}}\frac{[\mathrm{IGP}]}{K_{\mathrm{Glu,IGP}}}\frac{[\mathrm{Glu}]}{K_{\mathrm{Glu}}}. \quad \text{(A3)}$$

It should be noted that the equilibrium constant of the glutaminase reaction does not depend on what of the three forms of the enzyme (free enzyme, enzyme bound to ProFAR or to IGP) catalyzes the reaction; consequently, the following relationships are correct:

$$K^{\mathrm{g}}_{\mathrm{eq}} = \frac{K^{\mathrm{g}}_{\mathrm{Glu}}}{K_{\mathrm{Gln}}}\frac{k_2}{k_{-2}} = \frac{K^{\mathrm{g}}_{\mathrm{ProFAR,Glu}}}{K_{\mathrm{ProFAR,Gln}}}\frac{k_4}{k_{-4}} = \frac{K^{\mathrm{g}}_{\mathrm{IGP,Glu}}}{K_{\mathrm{IGP,Gln}}}\frac{k_3}{k_{-3}}, \quad \text{(A4)}$$

which make it possible to express k_{-3} and k_{-4} via k_2 and k_{-2}:

$$k_{-4} = k_4 \frac{K_{\mathrm{ProFAR,Gln}}}{K^{\mathrm{g}}_{\mathrm{ProFAR,Glu}}}\frac{K^{\mathrm{g}}_{\mathrm{Glu}}}{K_{\mathrm{Gln}}}\frac{k_2}{k_{-2}},$$

$$k_{-3} = k_3 \frac{K_{\mathrm{IGP,Gln}}}{K^{\mathrm{g}}_{\mathrm{IGP,Glu}}}\frac{K^{\mathrm{g}}_{\mathrm{Glu}}}{K_{\mathrm{Gln}}}\frac{k_2}{k_{-2}}. \quad \text{(A5)}$$

Substitution of (A3) and (A5) into Eqs. (8) and (9) of the main text gives the following equations for the rates of the synthetase and glutaminase reactions:

$$v_s = \frac{[\mathrm{HisHF}]}{\Delta}\left(k_1\frac{[\mathrm{Gln}]}{K_{\mathrm{PRFAR,Gln}}}\frac{[\mathrm{PRFAR}]}{K_{\mathrm{PRFAR}}} - k_{-1}\frac{[\mathrm{AICAR}]}{K_{\mathrm{Glu,IGP,AICAR}}}\frac{[\mathrm{IGP}]}{K_{\mathrm{Glu,IGP}}}\frac{[\mathrm{Glu}]}{K_{\mathrm{Glu}}}\right),$$

$$v_g = \frac{[\mathrm{HisHF}]}{\Delta}\left(\frac{k_2}{K_{\mathrm{Gln}}} + \frac{k_3}{K_{\mathrm{IGP,Gln}}}\frac{[\mathrm{IGP}]}{K_{\mathrm{IGP}}} + \frac{k_4}{K_{\mathrm{ProFAR,Gln}}}\frac{[\mathrm{ProFAR}]}{K_{\mathrm{ProFAR}}}\right)\left([\mathrm{Gln}] - \frac{k_{-2}}{k_2}\frac{K_{\mathrm{Gln}}}{K^{\mathrm{g}}_{\mathrm{Glu}}}[\mathrm{Glu}]\right).$$

APPENDIX 2

Let (B1) be the rate equation of the enzyme functioning in which parameters of the catalytic cycle (the rate and dissociation constants of certain stages) are the terms:

$$v = [\mathrm{E}]_{\mathrm{tot}} \cdot f(\mathrm{S}_1, ..., \mathrm{S}_i, ..., \mathrm{S}_n, \mathrm{P}_1, ..., \mathrm{P}_j,$$
$$..., \mathrm{P}_m, \mathrm{M}_1, ..., \mathrm{M}_h, ..., \mathrm{M}_q), \quad \text{(B1)}$$

where $[\mathrm{E}]_{\mathrm{tot}}$ is the total enzyme concentration and S_i (i = 1, ..., n), P_j (j = 1, ..., m), and M_h (h = 1, ..., q) are concentrations of the substrates, products, and modifiers (inhibitors and activators), respectively. Using biochemical definitions of conventional parameters of enzymatic kinetics (the Michaelis constants of the substrates and products, the equilibrium constants, the maximal number of enzyme cycles in forward reaction in the presence and in the absence of activators and inhibitors), let us find how to express the kinetic parameters via parameters of the catalytic cycle. By definition, the maximal number of the enzyme cycles in the forward reaction is the ratio of the maximal rate of enzyme functioning to the total enzyme concentration at saturating concentrations of all substrates and zero concentrations of all products and modifiers. This means that for calculation of the maximal number of enzyme cycles in the forward reaction, the following expression should be used:

$$k^{\mathrm{f}}_{\mathrm{cat}} = \lim_{\substack{\mathrm{S}_i \to \infty, i=1,...,n \\ \mathrm{P}_j = 0, j=1,...,m \\ \mathrm{M}_h = 0, h=1,...,q}} f(\mathrm{S}_1,...,\mathrm{S}_n,\mathrm{P}_1,...,\mathrm{P}_m,\mathrm{M}_1,...,\mathrm{M}_q). \quad \text{(B2)}$$

Analogously the maximal number of enzyme cycles at the saturating concentration of modifier (inhibitor or activator) M_r can be calculated:

$$k^{\mathrm{f,M_r}}_{\mathrm{cat}} = \lim_{\substack{\mathrm{S}_i \to \infty, i=1,...,n \\ \mathrm{M}_r \to \infty \\ \mathrm{P}_j = 0, j=1,...,m \\ \mathrm{M}_h = 0, h=1,...r-1,r+1,...,q}} f(\mathrm{S}_1,...,\mathrm{S}_n,\mathrm{P}_1,...,\mathrm{P}_m,\mathrm{M}_1,...,\mathrm{M}_q). \quad \text{(B3)}$$

The equilibrium constant can be found from the following expression:

$$K_{\mathrm{eq}} = \prod_{j=1}^{m}\mathrm{P}^{\mathrm{eq}}_j \Big/ \prod_{i=1}^{n}\mathrm{S}^{\mathrm{eq}}_i, \quad \text{(B4)}$$

where the equilibrium concentrations of the substrates $\mathrm{S}^{\mathrm{eq}}_i$ (i = 1, ..., n) and products $\mathrm{P}^{\mathrm{eq}}_j$ (j = 1, ..., m) are solutions of the following equation:

$$f(\mathrm{S}_1^{\mathrm{eq}}, ..., \mathrm{S}_i^{\mathrm{eq}}, ..., \mathrm{S}_n^{\mathrm{eq}}, \mathrm{P}_1^{\mathrm{eq}}, ..., \mathrm{P}_j^{\mathrm{eq}}, ..., \mathrm{P}_m^{\mathrm{eq}},$$
$$\mathrm{M}_1, ..., \mathrm{M}_h, ..., \mathrm{M}_q) = 0. \quad \text{(B5)}$$

By definition, the Michaelis constant of the enzyme with some substrate is the concentration of the considered substrate at which the rate of the enzyme functioning is a half of its maximal rate under the conditions when the products and modifiers (inhibitors and activators) are absent and concentrations of all other substrates are saturating. In accord with this definition, $K_{\mathrm{m,St}}$, the Michaelis constant of substrate S_t, is a solution of the following equation:

$$\frac{k^{\mathrm{f}}_{\mathrm{cat}}}{2} = F_{\mathrm{S_t}}\left(K_{\mathrm{m,S_t}}\right),$$

where

$$F_{S_t}(S_t) = \lim_{\substack{S_t \to \infty, i=1,\ldots,t-1,t+1,\ldots,n \\ P_j=0, j=1,\ldots,m \\ M_h=0, h=1,\ldots,q}} f(S_1,\ldots,S_n, P_1,\ldots,P_m, M_1,\ldots,M_q) . \quad \text{(B6)}$$

Analogously, the Michaelis constant of substrate S_t at the saturating concentration of modifier (inhibitor or activator) M_r:

$$\frac{k_{cat}^{f,M_r}}{2} = F_{S_t}^{M_r}\left(K_{m,S_t}^{M_r}\right),$$

where

$$F_{S_t}^{M_r}(S_t) = \lim_{\substack{S_t \to \infty, i=1,\ldots,t-1,t+1,\ldots,n \\ M_r \to \infty \\ P_j=0, j=1,\ldots,m \\ M_h=0, h=1,\ldots,r-1,r+1,\ldots,q}} f(S_1,\ldots,S_n, P_1,\ldots, P_m, M_1,\ldots,M_q) \quad \text{(B7)}$$

and the Michaelis constant of product P_t are calculated:

$$\frac{k_{cat}^{b}}{2} = F_{P_t}\left(K_{m,P_t}\right),$$

where

$$F_{P_t}(P_t) = \lim_{\substack{P_j \to \infty, j=1,\ldots,t-1,t+1,\ldots,m \\ S_i=0, i=1,\ldots,n \\ M_h=0, h=1,\ldots,q}} \left(-f(S_1,\ldots,S_n, P_1,\ldots,P_m, M_1,\ldots,M_q)\right). \quad \text{(B8)}$$

In this expression, the maximal number of enzyme cycles in the reverse reaction, k_{cat}^{b}, is calculated as follows:

$$k_{cat}^{b} = \lim_{\substack{P_j \to \infty, j=1,\ldots,m \\ S_i=0, i=1,\ldots,n \\ M_h=0, h=1,\ldots,q}} \left(-f(S_1,\ldots,S_n, P_1,\ldots,P_m, M_1,\ldots,M_q)\right). \quad \text{(B9)}$$

Applying formulae (B1)-(B9) to the rate equations of the synthetase and glutaminase reactions (Eqs. (10) of the main text), we deduced the following relationships between the parameters of the catalytic cycle and the conventional parameters of enzymatic kinetics:

$$k_{cat}^{s} = k_1 ;$$

$$K_{m,Gln}^{s} = K_{PRFAR,Gln} ;$$

$$K_{m,PRFAR}^{s} = K_{PRFAR}\frac{K_{PRFAR,Gln}}{K_{Gln}} ;$$

$$K_{eq}^{s} = \frac{k_1}{k_{-1}}\frac{K_{Glu,IGP,AICAR}K_{Glu,IGP}K_{Glu}}{K_{PRFAR,Gln}K_{PRFAR}} ;$$

$$K_{m,AICAR}^{s} = K_{Glu,IGP,AICAR}\left(1+\frac{K_{Glu,IGP}K_{Glu}}{K_{IGP,Glu}^{g}K_{IGP}}\right);$$

$$K_{m,Glu}^{s} = K_{Glu,IGP,AICAR}\frac{K_{Glu,IGP}K_{Glu}}{K_{IGP,AICAR}K_{IGP}} ;$$

$$K_{m,IGP}^{s} = K_{Glu,IGP,AICAR}\frac{K_{Glu,IGP}K_{Glu}}{K_{AICAR,Glu}K_{AICAR}} ;$$

$$k_{cat}^{g} = k_2 ;$$

$$K_{m,Gln}^{g} = K_{Gln} ;$$

$$K_{eq}^{g} = \frac{k_2}{k_{-2}}\frac{K_{Glu}^{g}}{K_{Gln}} ;$$

$$K_{m,Glu}^{g} = K_{Glu}\frac{K_{Glu}^{g}}{K_{Glu}+K_{Glu}^{g}} ;$$

$$k_{cat}^{g,IGP} = k_3 ;$$

$$K_{m,Gln}^{g,IGP} = K_{IGP,Gln} ;$$

$$K_{m,Glu}^{g,IGP} = K_{IGP,Glu}^{g} ;$$

$$k_{cat}^{g,ProFAR} = k_4 ;$$

$$K_{m,Gln}^{g,ProFAR} = K_{ProFAR,Gln} ;$$

$$K_{m,Glu}^{g,ProFAR} = K_{ProFAR,Glu}^{g} .$$

These relationships allow expressing parameters of the catalytic cycle via kinetic parameters:

$$k_1 = k_{cat}^{s} ;$$

$$K_{PRFAR,Gln} = K_{m,Gln}^{s} ;$$

$$k_2 = k_{cat}^{g} ;$$

$$K_{Gln} = K_{m,Gln}^{g} ;$$

$$k_3 = k_{cat}^{g,IGP} ;$$

$$K_{IGP,Gln} = K_{m,Gln}^{g,IGP} ;$$

$$K_{IGP,Glu}^{g} = K_{m,Glu}^{g,IGP} ;$$

$$k_4 = k_{cat}^{g,ProFAR} ;$$

$$K_{ProFAR,Gln} = K_{m,Gln}^{g,ProFAR} ;$$

$$K_{ProFAR,Glu}^{g} = K_{m,Glu}^{g,ProFAR} ;$$

$$K_{\mathrm{PRFAR}} = K^{\mathrm{s}}_{\mathrm{m,PRFAR}} \frac{K^{\mathrm{g}}_{\mathrm{m,Gln}}}{K^{\mathrm{s}}_{\mathrm{m,Gln}}};$$

$$K_{\mathrm{Glu,IGP,AICAR}} = \frac{K^{\mathrm{s}}_{\mathrm{m,AICAR}} K^{\mathrm{g,IGP}}_{\mathrm{m,Glu}} K_{\mathrm{IGP}}}{K_{\mathrm{Glu}} K_{\mathrm{Glu,IGP}} + K_{\mathrm{IGP}} K^{\mathrm{g,IGP}}_{\mathrm{m,Glu}}};$$

$$K^{\mathrm{g}}_{\mathrm{Glu}} = K^{\mathrm{g}}_{\mathrm{m,Glu}} \frac{K_{\mathrm{Glu}}}{K_{\mathrm{Glu}} - K^{\mathrm{g}}_{\mathrm{m,Glu}}}, K_{\mathrm{Glu}} > K^{\mathrm{g}}_{\mathrm{m,Glu}};$$

$$k_{-2} = \frac{k^{\mathrm{g}}_{\mathrm{cat}}}{K^{\mathrm{g}}_{\mathrm{eq}} K^{\mathrm{g}}_{\mathrm{m,Gln}}} \frac{K^{\mathrm{g}}_{\mathrm{m,Glu}} K_{\mathrm{Glu}}}{K_{\mathrm{Glu}} - K^{\mathrm{g}}_{\mathrm{m,Glu}}}, K_{\mathrm{Glu}} > K^{\mathrm{g}}_{\mathrm{m,Glu}};$$

$$K_{\mathrm{IGP,AICAR}} = \frac{K^{\mathrm{s}}_{\mathrm{m,AICAR}} K^{\mathrm{g,IGP}}_{\mathrm{m,Glu}} K_{\mathrm{Glu}} K_{\mathrm{Glu,IGP}}}{K^{\mathrm{s}}_{\mathrm{m,Glu}} \left(K_{\mathrm{Glu}} K_{\mathrm{Glu,IGP}} + K_{\mathrm{IGP}} K^{\mathrm{g,IGP}}_{\mathrm{m,Glu}} \right)};$$

$$K_{\mathrm{AICAR,Glu}} = \frac{K^{\mathrm{s}}_{\mathrm{m,AICAR}} K^{\mathrm{g,IGP}}_{\mathrm{m,Glu}} K_{\mathrm{Glu}} K_{\mathrm{Glu,IGP}} K_{\mathrm{IGP}}}{K^{\mathrm{s}}_{\mathrm{m,IGP}} K_{\mathrm{AICAR}} \left(K_{\mathrm{Glu}} K_{\mathrm{Glu,IGP}} + K_{\mathrm{IGP}} K^{\mathrm{g,IGP}}_{\mathrm{m,Glu}} \right)};$$

$$k_{-1} = \frac{k^{\mathrm{s}}_{\mathrm{cat}}}{K^{\mathrm{s}}_{\mathrm{eq}} K^{\mathrm{s}}_{\mathrm{m,PRFAR}} K^{\mathrm{g}}_{\mathrm{m,Gln}}} \frac{K^{\mathrm{s}}_{\mathrm{m,AICAR}} K^{\mathrm{g,IGP}}_{\mathrm{m,Glu}} K_{\mathrm{Glu}} K_{\mathrm{Glu,IGP}} K_{\mathrm{IGP}}}{K_{\mathrm{Glu}} K_{\mathrm{Glu,IGP}} + K_{\mathrm{IGP}} K^{\mathrm{g,IGP}}_{\mathrm{m,Glu}}}.$$

Substitution of these relationships into Eqs. (10) of the main text gives Eqs. (11).