MATHEMATICAL MODELING OF REGULATION
OF ESCHERICHIA COLI PURINE BIOSYNTHESIS
PATHWAY ENZYMATIC REACTIONS

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SUMMARY

Motivation: Development of mathematical models that adequately describe molecular-genetic mechanisms is one of the main tasks in modern bioinformatics. Not only should a model be sensitive to the particulars of enzyme reactions underlying cell life activity, but also to regulation exerted on enzyme activity by metabolites and specialized cell proteins.

Results: The gene network for purine biosynthesis in E. coli has been reconstructed using the GeneNet system. The elementary mathematical models for enzyme reactions have been developed using Hill’s generalized functions. We herein give a detailed description of the mathematical models (1) for the biosynthetic IMP\(^a\) dehydrogenase-catalyzed reaction and (2) for the reaction catalyzed by Adenylosuccinate synthetase (AdSS); the models include enzyme activity regulation.

Availability: The models are available on request; the gene network is available at http://wwwmgs.bionet.nsc.ru/mgs/gnw/genenet/viewer/index.shtml.

INTRODUCTION

Biosynthesis of the purines AMP and GMP in E. coli is a many-staged process supported by a complex network of enzymes (Table 1). Some of the genes that encode these enzymes, are arranged into operons (purF, purHD, purMN, purEK, guaBA, purB), others are located in single cistrons (purT, purL, purC, purA, guaA). Many of the genes in

\(^a\) The abbreviations used are: ADP, Adenosine diphosphate; AdSS, Adenylosuccinate synthetase; AICAR, 5-Phosphate-ribosyl-5-amino-4-imidazole carboxamide; AIR, Aminoimidazole ribotide; AMP, Adenosine monophosphate; ASP, Aspartate; ASUC, Adenilsuccinate; ATP, Adenosine triphosphate; CAIR, 5-Phosphoribosyl-5-aminoimidazole-4-carboxylate; CMP, Cytidine monophosphate; CO2, Carbon dioxide; dAMP, DeoxyAMP; dGMP, DeoxyGMP; GAR, Glycinamide ribonucleotide; GDP, Guanosine diphosphate; GLN, glutamine; GMP, Guanosine monophosphate; GDP, Guanosine diphosphate; GTP, Guanosine triphosphate; FGAM, 5-Phosphoribosyl-n-formylglycinamidine; FGAR, N-Formyl-GAR; FOR, Formate; FTHF, 10-Formyltetrahydrofolate; FUM, Fumarate; GLN, Glutamine; GLU, Glutamate; GLY, Glycine; IMP, Inosine monophosphate; NAD, Nicotinamide adenine dinucleotide; NADH, NAD reduced; NADP, NAD phosphate; NADPH, NAD phosphate reduced; NCAIR, N5-carboxyaminoimidazole ribonucleotide; NH3, Ammonia; PI, Phosphate; PPI, Pyrophosphate; PRAM, Phosphate-ribosyl amine; PRFICA, Phosphoribosylformamido-carboxamide; PRPP, Phosphoribosylpyrophosphate; SAICAR, 5-Phosphoribosyl-4-(N-succinocarboxamido)-5-amino-imidazole; SUCC, Succinate; THF, Tetrahydrofolate; UMP, uridine monophosphate; XMP, Xanthosine monophosphate.
the network for purine biosynthesis are controlled by the protein PurR (which is encoded by the gene purR) and its co-repressors hypoxanthine and guanine. Besides, the operon guaBA is repressed by GMP and the protein DnaA, and is activated by CRP (the cAMP receptor protein) and AMP. AMP and GMP have opposing effects on the operon guaC. The operon guaC, too, is repressed by GLN. Some other low-molecular-weight items participate in the regulation of gene expression and enzyme activity in this gene network.

<table>
<thead>
<tr>
<th>№</th>
<th>Enzyme names</th>
<th>Genes</th>
<th>Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Amidophosphoribosyl transferase</td>
<td>purF</td>
<td>PRPP + GLN -&gt; PPI + GLU + PRAM</td>
</tr>
<tr>
<td>2</td>
<td>Phosphoribosylamine-glycine ligase</td>
<td>purD</td>
<td>PRAM + ATP + GLY &lt;-&gt; ADP + PI + GAR</td>
</tr>
<tr>
<td>3</td>
<td>Phosphoribosylglycinamide formyltransferase</td>
<td>purN</td>
<td>GAR + FTHF -&gt; THF + FGAR</td>
</tr>
<tr>
<td>4</td>
<td>GAR transformylase T</td>
<td>purT</td>
<td>GAR + FOR + ATP -&gt; ADP + PI + FGAR</td>
</tr>
<tr>
<td>5</td>
<td>Phosphoribosylformylglycinamide synthetase</td>
<td>purL</td>
<td>FGAR + ATP + GLN -&gt; GLU + ADP + PI + FGAM</td>
</tr>
<tr>
<td>6</td>
<td>Phosphoribosylformylglycinamide cyclo-ligase</td>
<td>purM</td>
<td>FGAM + ATP -&gt; ADP + PI + AIR</td>
</tr>
<tr>
<td>7</td>
<td>Phosphoribosylaminomimidazole carboxylase 1</td>
<td>purK</td>
<td>AIR + CO2 + ATP &lt;-&gt; NCAIR + ADP + PI</td>
</tr>
<tr>
<td>8</td>
<td>Phosphoribosylaminomimidazole carboxylase 2</td>
<td>purE</td>
<td>NCAIR &lt;-&gt; CAIR</td>
</tr>
<tr>
<td>9</td>
<td>Phosphoribosylaminomimidazole- succinocarboxamide synthetase</td>
<td>purC</td>
<td>CAIR + ATP + ASP &lt;-&gt; ADP + PI + SAICAR</td>
</tr>
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<td>5'-Phosphoribosyl-4-(N-succinocarboxamide)-5-aminoimidazole lyase</td>
<td>purB</td>
<td>SAICAR &lt;-&gt; FUM + AICAR</td>
</tr>
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<td>11</td>
<td>AICAR transformylase</td>
<td>purH</td>
<td>AICAR + FTHF &lt;*&gt; THF + PRFICA</td>
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<tr>
<td>12</td>
<td>IMP cyclohydrase</td>
<td>purH</td>
<td>PRFICA &lt;*&gt; IMP</td>
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<tr>
<td>13</td>
<td>Adenylosuccinate synthetase</td>
<td>purA</td>
<td>IMP + GTP + ASP -&gt; GDP + PI + ASUC</td>
</tr>
<tr>
<td>14</td>
<td>Adenylosuccinate lyase</td>
<td>purB</td>
<td>ASUC &lt;*&gt; FUM + AMP</td>
</tr>
<tr>
<td>15</td>
<td>IMPD, IMP dehydrogenase</td>
<td>guaB</td>
<td>IMP + NAD -&gt; NADH + XMP</td>
</tr>
<tr>
<td>16</td>
<td>GMP synthase</td>
<td>guaA</td>
<td>XMP + ATP + GLN -&gt; GLU + AMP + PPI + GMP</td>
</tr>
<tr>
<td>17</td>
<td>GMP reductase</td>
<td>guaC</td>
<td>GMP + NADPH -&gt; NADP + IMP + NH3</td>
</tr>
</tbody>
</table>

IMP dehydrogenase of _E. coli_ (IMPD; EC 1.2.1.14) catalyzes the NAD⁺-dependent conversion of IMP into XMP. IMPD attracts scientists as a potential target in developing antimicrob, antitumor and immune suppressing drugs. This is a tetramer, which consists of identical subunits and is competitively inhibited by GMP. The enzyme activity of IMPD is enhanced by K⁺: the mechanism of this enhancement is not yet absolutely clear, however, it is proposed that K⁺ facilitates the binding of the enzyme to NAD (Gilbert _et al._, 1979; Kerr _et al._, 2000).

The enzyme adenylosuccinate synthetase (AdSS; GDP-forming IMP: L-aspartate ligase, EC 6.3.4.4) catalyzes the reaction of conversion of IMP to ASUC in the presence of Mg²⁺. There are many nucleotides that inhibit AdSS: AMP is an inhibitor competitive with respect to IMP; ASUC - to IMP; dGMP - to IMP; GMP - to GTP; GDP - with respect to GTP, which in part explains a gradual decrease in the rates of formation ASUC in the solutions in which GTP is not reduced. Weak inhibitory effects are also produced by dAMP, CMP and UMP (Wyngaarden _et al._, 1963). Mathematical models for the reaction catalyzed by AdSS were proposed in a variety of works: in 1969, Rudolph proposed an equation which included the effect of one inhibitor – that equation contained 11 parameters; in 1979, Stayton proposed a slightly different equation for one inhibitor – that equation contained 12 parameters (Stayton, Fromm, 1979); in 1995, Kang proposed an equation which related the reaction rate and ASP, which is one of the substrates, and Mg²⁺ ions – that equation contained 4 parameters.
We have reconstructed the gene network for purine biosynthesis and developed elementary mathematical models for enzyme reactions using generalized Hill functions. Herein we give a detailed description of the models (1) for the biochemical reaction catalyzed by IMPD and regulated by GMP and $K^+$ ions, and (2) propose rather a simple model for the reaction catalyzed by AdSS, considering the effects of five inhibitors and $Mg^{2+}$ ions and containing 10 parameters.

**METHODS AND ALGORITHMS**

The reconstruction of the gene network for purine biosynthesis was performed using the GeneNet system (Ananko et al., 2005). The biochemical reactions were modeled using Hill’s generalized functions (Likhoshvai et al., 2006).

**RESULTS**

The gene network for purine biosynthesis has been reconstructed using the GeneNet system (Fig. 1) and is accessible via the Internet. The number of the reconstructed gene network components, of which GeneNet is aware, is presented in Table 2.

![Figure 1. A fragment of the gene network for purine biosynthesis reconstructed using the GeneNet system: regulation of the expression of the guaB operon and the enzyme activity of IMPD. Squares denote low-molecular-weight compounds; circles, monomeric proteins; double oval, the dimeric protein; triple oval, the multimer; broken line, RNA; arrowed rectangle, the operon; arrowed dash lines, transcription and translation; other arrows, reactions and regulatory actions.](image)

| Table 2. The number of items collected as data on the gene network for purine biosynthesis |
|--------------------------------------|------|------|------|------|------|------|------|
| Item                                | Protein | RNA | Gene | Operon | Reaction, regulation | Inorganic substance | Protein-repressor | Literature source |
| Quantity                           | 33 | 12 | 15 | 11 | 126 | 42 | 3 | 140 |

Using generalized Hill functions, mathematical models have been developed for the enzyme reactions listed in Table 1. Verification of the model’s parameters was performed using data in the Kinet database containing constants and dynamic data from published experimental works (Khleboboroda et al., this issue). The steady-state and dynamic characteristics of the biochemical reactions studied using the model were compared with the experimental data and a good agreement was obtained. For example, knowing how GMP and $K^+$ ions affect the IMPD-catalyzed reaction ($K^+$ ions enhance enzyme activity, GMP inhibits it competitively with respect to IMP), the reaction rate can be written in a generalized form as follows:
Modelling of molecular genetic systems in bacterial cell

\[ V = E_0 \cdot k_0 \cdot \left( \frac{IMP \cdot K_{mIMP}}{1 + IMP \cdot K_{mIMP} + GMP \cdot K_{mGMP}} \right) \cdot \left( \frac{NAD \cdot K_{mNAD}}{1 + NAD \cdot K_{mNAD}} \right) \cdot \frac{1 + K^+ / k_1}{1 + K^+ / k_2} \]

where \( V \) is the XMP synthesis rate; \( E_0 \) is the IMPD concentration; \( k_0 \) is the rate constant of enzyme; \( IMP, NAD, GMP \) and \( K^+ \) are \( IMP, NAD, GMP \) and \( K^+ \) concentrations; \( K_{mIMP}, K_{mNAD} \) are the Michaelis-Menten constants for the respective substrates; \( K_{mGMP} \) is the constant of GMP inhibiting the reaction; \( k_1, k_2 \) are the constants of \( K^+ \) ions affecting enzyme activity.

How the model (1) was fit to experimental data on the effect of the \( IMP, NAD, GMP \) and \( K^+ \) concentrations on the \( XMP \) biosynthesis rates is presented in Fig. 2. Fitting resulted in the following values for the parameters: \( k_0 = 0.18 \) 1/sec, \( K_{mIMP} = 0.0115 \) mM, \( K_{mNAD} = 0.334 \) mM, \( K_{mGMP} = 0.08 \) mM, \( k_1 = 0.18 \) mM, \( k_2 = 5 \) mM.

Figure 2. XMP synthesis rates: \( a \) – depending on IMP concentrations with 10mM NAD and without \( K^+ \) (solid line and \( \mathbb{O} \): 0 GMP; dotted line and \( \mathbb{D} \): 0.1mM GMP; broken lines and \( \mathbb{R} \): 0.2mM GMP); \( b \) – depending on the concentration of \( K^+ \) ions (1mM IMP, 2.5 mM NAD, 0 GMP). \( \mathbb{O}, \mathbb{D}, \mathbb{R} \) indicate experimental data from Gilbert et al. , 1979; B, Kerr et al., 2000); curves indicate results obtained from the model (1).

Second example – is the model of reaction, catalyzed by AdSS. This enzyme is inhibited by GMP, GDP, AMP, ASUC and SUCC, and requires the presence of Mg\(^{2+}\) ions. Knowing how these effectors work, the reaction rate can be written in a generalized form as follows:

\[ V = V_{\text{max}} \cdot \left( \frac{GTP \cdot IMP \cdot ASP}{K_{mGTP} \cdot K_{mIMP} \cdot K_{mASP}} \right) \cdot \left( \frac{Mg^{2+}}{K_{mMg}} \right), \]

where \( V_{\text{max}} \) is the maximum reaction rate; \( GTP, IMP, ASP \) are the concentrations of the corresponding substrates; \( GMP, GDP, AMP, ASUC, SUCC \) are the concentrations of the corresponding inhibitors; \( Mg^{2+} \) is the concentration of Mg\(^{2+}\) ions; \( K_{mGTP}, K_{mIMP}, K_{mASP} \) are the Michaelis-Menten constant for the respective substrates; \( K_{mMg} \) is the Michaelis-Menten constant for Mg\(^{2+}\) ions; \( K_{GMP}, K_{GDP}, K_{AMP}, K_{ASUC}, K_{SUCC} \) are the constants of the efficiency of reaction inhibition by corresponding substances.

The model’s parameters were verified against 61 curves from literature (Wyngaarden et al., 1963; Rudolph, Fromm, 1969; Kang, Fromm, 1995). Rate constant of the enzyme.
AdSS is 15600 1/s (Dong et al., 1991); however, since no enzyme concentrations were indicated, we calculated the value for $V_{\text{max}}$ using our model.

The results of calculations using our model and the experimental data (Rudolph, Fromm, 1969), to which the fitting of the model was performed, are presented in Fig. 3. The parameter values inferred from curves were as follows (Fig. 3a, b, c): $V_{\text{max}} = 1.35 \times 10^{-3}$ mM/min, $K_{mGTP} = 0.023$ mM, $K_{mIMP} = 0.02$ mM, $K_{mASP} = 0.3$ mM.

**Figure 3** Relationships between the reaction rate ($V$) and the concentrations of GTP (a), IMP (b) and ASP (c), respectively. O, □, ◊ and × represent experimental data from Rudolph et al. (1969); lines are the results of calculations using our mathematical model (2).

The effect of SUCC has been studied in detail (Rudolph, Fromm, 1969); we examined its effect on all the three substrates. The modeling results and experimental data are presented in Fig. 4. Using the model, the value of the constant $K_{\text{SUCC}}$, is 8 mM. As can be seen from Fig. 4a, there is an inconsistency between modeling results and experimental evidence. To do away with this inconsistency, however, is easy, by introducing to the model a correction factor (a multiplier equal to 0.74), which in fact implies a reduced concentration of the enzyme compared to the experimental data shown in the other curves (calculations not shown).

**Figure 4** Relationships between the reaction rate ($V$) and the concentrations of GTP (a), IMP (b) and ASP (c), respectively. a – SUCC concentrations were (solid line and O) 50 mM; (dashed line and □) 25 mM; (dotted line and ◊) 12.5 mM; (lowest line and ×) 0. b – SUCC concentrations were (solid line and O) 20 mM; (dashed line and □) 10 mM; (dotted line and ◊) 0. c – SUCC concentrations were (solid line and O) 20 mM; (dashed line and □) 10 mM; (dotted line and ◊) 0. O, □, ◊, × represent experimental data from Rudolph, Fromm (1969); lines are the results of calculations using our mathematical model (2).
The modeling results and experimental data on GDP effects are presented in Fig. 5a. Using the model, the value of the constant $K_{GDP}$ is $8 \cdot 10^{-3}$ mM. The effects of ASUC on the reaction substrates IMP and ASP have been examined; the results, both experimental and modeling, are presented in Fig. 5b, c. Using the model, the value of the constant $K_{ASUC}$ is $7.5 \cdot 10^{-3}$ mM.

![Figure 5. Relationships between the reaction rate (V) and the concentrations of GTP (a), IMP (b) and ASP (c), respectively. $\bigcirc$, $\square$, $\triangle$, $\times$ represent experimental data from Rudolph, Fromm (1969); lines are the results of calculations using our mathematical model (2).](image)

The constant $K_{mMg}$ was verified against data published by Kang, Fromm (1995) and is 0.08 mM (Fig. 6a). However, in so doing, we had to reduce $K_{mASP}$ two-fold, to 0.17 mM and introduced to the model a correction factor (a multiplier equal to 5000), which increases the amount of the enzyme. The other parameters were absolutely consistent with those experimental data.

![Figure 6. Relationships between the reaction rate (V) and: concentrations of Mg$^{2+}$ ions at varying concentrations of ASP (a) or concentrations of ASP under different conditions (b). $\bigcirc$, $\square$, $\triangle$, $\times$ represent experimental data (a – Kang, Fromm, 1995; b – Wyngaarden et al., 1963); lines are the results of calculations using our mathematical model.](image)
Data from the work by Wyngaarden et al. (1963) were used as the control experiments. Analyzing the curves in this paper, an apparent inconsistency revealed. It is possible that the authors were dealing with different amounts of the enzyme in different experiments. This assumption was supported by introduction of a coefficient like the ones for the curves in Fig. 4a. A comparison of modeling results and experimental data (Wyngaarden et al., 1963) is presented in Fig. 6b, lines 4 and 5. Based on the work by Wyngaarden et al. (1963), we calculated the constants of GMP and AMP effects (Fig. 6b, lines 1 and 2): $K_{GMP} = 0.024$ mM, $K_{AMP} = 0.01$ mM. However, these estimates may suffer due to lack of concordance between the experimental data.

Note that the discrepancies revealed at fitting the parameters could in part be explained by different temperatures at which the different authors conducted their experiments: Rudolph, Fromm (1969), at 28 °C, Wyngaarden et al. (1963), at 25 °C, and Kang, Fromm (1995), at 22 °C. However, in the present work we did not look at temperature as a factor.

**DISCUSSION**

Using Hill’s generalized functions, mathematical models have been developed for the reactions involved in purine biosynthesis (listed in Table 1). The models capture the effects of all the regulatory proteins and low-molecular-weight compounds. The parameters were introduced to the models using published experimental data.

We herein present the models (1) for the dependence of XMP biosynthesis rates on the concentration of the reaction substrates IMP, NAD, inhibitor GMP and the activator $K^+$; (2) for the reaction catalyzed by the AdSS, which includes relationships between the reaction rate, the concentrations of three substrates (GTP, IMP and ASP), the effects of five inhibitors (GMP, GDP, AMP, ASUC and SUCC) and Mg$^{2+}$. Fitting of the model’s parameters was performed on the basis of experimental data in published literature. A methodical nuisance that came from the lack of concordance between data in different publications published was dealt with by introduction of correction coefficients. The adequacy of the model was ensured by comparing the theoretical calculations and the experimental data from the literature sources that were not being used while the fitting procedure was under way.

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