GENE NETWORK RECONSTRUCTION AND MATHEMATICAL MODELING OF SALVAGE PATHWAYS: REGULATION OF ADENINE PHOSPHORIBOSYLTRANSFERASE ACTIVITY BY STRUCTURALLY SIMILAR SUBSTRATES

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SUMMARY

Motivation: Development of an *in silico* cell, a computer resource for modeling and analysis of physiological processes is an urgent task of systems biology and computational biology. Mathematical modeling of the genetic regulation of cell metabolism pathways, in particular, salvage pathways, is an important problem to be solved as part of this line of work.

Results: By using the GeneNet technology, we reproduced the gene network of the regulation of salvage pathways in the *E. coli* cell. Mathematical models were constructed by the method of generalized Hill functions to describe the efficiency of enzyme systems and regulation of expression of genes coding for these enzymes.

Availability: The diagram of the gene network is available through the GeneNet viewer at http://wwwmgs.bionet.nsc.ru/mgs/gnw/genenet/viewer/index.shtml. Models are available on request.

INTRODUCTION

Salvage pathways are the metabolic pathways used by *Escherichia coli* for synthesis and conversion of adenine, hypoxanthine, guanine, xanthine, and their nucleosides and pyrimidine ribo- and deoxyribonucleotides.

The gene network of regulation of salvage pathways in the *E. coli* cell was reconstructed. Mathematical models of enzymatic reactions were constructed. A database storing experimental data on the behavior of components of this gene network was developed (Khlebodarova *et al.*, 2006). Parameters of the models were determined by numerical simulation. The results of calculation of steady-state properties and behavior of the components of the molecular system derived from the models are in agreement with experimental evidence.

METHODS AND ALGORITHMS

The gene network of salvage pathways was reconstructed with the use of the GeneNet technology (Ananko *et al.*, 2005), allowing accumulation and presentation of data on the

structure and function of molecular systems. Mathematical models of the regulation of gene expression and efficiency of enzyme systems were constructed by the method of generalized Hill functions (Likhoshvai, Ratushny, 2006).

RESULTS

The gene network of regulation of salvage pathways was reconstructed with the use of the GeneNet technology (Ananko *et al.*, 2005). In addition to *de novo* synthesis, purines, pyrimidines and their nucleosides can be formed in the cells via salvage reactions. The salvage pathways of *E. coli* involve 33 enzymes catalyzing 85 reactions (Table 1).

Table 1. Enzymatic reactions present in the salvage pathway network

Enzyme	Gene	Reaction	Alternative substrate	EC
Adenylate kinase	*adk*	GTP + AMP ↔ ADP + GDP	ATP, ITP, DAMP	2.7.4.3
Guanylate kinase	*gmk*	GMP + ATP ↔ GDP + ADP	DGMP	2.7.4.8
Nucleoside-diphosphate kinase	*ndk*	GDP + ATP ↔ GTP + ADP	UDP, CDP, DGDP, DUDP, DCDP, DADP, DTDP	2.7.4.6
AMP Nucleosidse	*amn*	AMP → AD + R5P		3.2.2.4
Adenosine deaminase	*add*	ADN → INS + NH3	DA	3.5.4.4
Adenine deaminase	*yicP*	AD → NH3 + HYXN		3.5.4.2
Inosine/ Guanosine kinase	*gsk*	INS + ATP → IMP + ADP	GSN	2.7.1.73
Adenine phosphoribosyltransferase	*apt*	AD + PRPP → PPI + AMP		2.4.2.7
Xanthine-guanine phosphoribosyltransferase	*gpt*	XAN + PRPP → XMP + PPI	GN, HYXN	2.4.2.22
Hypoxanthine phosphoribosyltransferase	*hpt*	HYXN + PRPP → PPI + IMP	GN	2.4.2.8
Xanthosine phosphorylase	*xapA*	DIN + PI ↔ HYXN + DR1P	DA, DG, INS, ADN, GSN, XTSN	2.4.2.1
Purine nucleotide phosphorylase	*deoD*	DIN + PI ↔ HYXN + DR1P	DA, DG, INS, ADN, GSN	2.4.2.1
Uridine phosphorylase	*udp*	URI + PI ↔ URA + R1P		2.4.2.3
Thymidine/deoxyuridine phosphorylase	*deoA*	DU + PI ↔ URA + DR1P	DT	2.4.2.4
Cytidylate kinase	*cmk*	CMP + ATP ↔ ADP + CDP	UMP, DCMP	2.7.4.14
dTMP kinase	*tmk*	DTMP + ATP ↔ ADP + DTDP		2.7.4.9
Uridylate kinase	*pyrH*	UMP + ATP ↔ UDP + ADP	DUMP	2.1.4.-
Uracil phosphoribosyltransferase	*upp*	URA + PRPP → UMP + PPI		2.4.2.9
Cytosine deaminase	*codA*	CYTS → URA + NH3		3.5.4.1
Uridine/Cytodine kinase	*udk*	URI + GTP → GDP + UMP	CYTD	2.7.1.48
Thymidine (deoxyuridine) kinase	*tdk*	DT + ATP → ADP + DTMP	DU	2.7.1.21
dCTP deaminase	*dcd*	DCTP → DUTP + NH3		3.5.4.13
Cytidine deaminase	*cdd*	DC → NH3 + DU	CYTD	3.5.4.5

Enzyme	Gene	Reaction	Alternative substrate	EC
5'-Nucleotidase	*ushA*	AMP → PI + ADN	GMP, IMP, XMP, UMP, CMP, DCMP, DGMP, DAMP, DTMP, DUMP	3.1.3.5
Ribonucleoside-diphosphate reductase	*nrdAB*	ADP + RTHIO → DADP + OTHIO	GDP, CDP, UDP	1.17.4.1
Ribonucleoside-triphosphate reductase	*nrdD*	ATP + RTHIO → DATP + OTHIO	GTP, CTP, UTP	1.17.4.2
Ribonucleoside-diphosphate reductase II	*nrdEF*	CDP + RTHIO → DCDP + OTHIO		
dUTP pyrophosphatase	*dut*	DUTP → PPI + DUMP		3.6.1.23
Thymidylate synthetase	*thyA*	DUMP + METTHF → DHF + DTMP		2.1.1.45
Nucleoside triphosphatase	*mutT*	GTP → GMP + PPI	DGTP	3.6.1.-
Deoxyguanosinetriphosphate triphophohydrolase	*dgt*	DGTP → DG + PPP	GTP	3.1.5.1

Table 1 shows the enzymatic reactions present in the gene network under consideration, names of enzymes catalyzing corresponding reactions, and names of genes coding for the enzymes. Table 2 summarizes the components of the salvage pathway network.

Table 2. Components of the salvage pathway network

Operon	RNA	Enzyme	Reaction	Inorganic substance	Repressor	Transcription factor	Reference
30	30	32	476	82	58	10	390

Escherichia coli possesses the ability to take up purine and pyrimidine nucleosides from the growth medium and use them as sources of nitrogen and carbon. Nucleoside phosphorylases catalyze the phosphorolytic cleavage of the nucleoside, thereby forming the free nucleotide base and (deoxy)ribose-1-phosphate. The base can be utilized by the purine or the pyrimidine salvage pathways, and the ribose-1-phosphate and the deoxyribose-1-phosphate can be converted to intermediates of the pentose phosphate shunt and of glycolysis, respectively. Of the four different nucleoside phosphorylases in *E. coli*, uridine phosphorylase (*udp*) and thymidine phosphorylase (*deoA*) are specific for pyrimidine nucleosides whereas purine nucleoside phosphorylase (*deoD*) and xanthosine phosphorylase (*xapA*) are specific for purine nucleosides. Purine nucleoside phosphorylase is important for the breakdown of all purine nucleosides and deoxynucleosides except xanthosine. Xanthosine phosphorylase (XapA), on the other hand, has specificity toward xanthosine and all other purine nucleosides and deoxynucleosides except adenosine and deoxyadenosine. Purine nucleoside phosphorylase (DeoD) is encoded by the last gene of the *deoCABD* operon. The regulation of these genes is complex and involves two repressors (CytR and DeoR) and an activator (cyclic AMP [cAMP] receptor protein-cAMP complex). Despite the action of two repressors, the *deo* genes are always expressed at a low basal level to ensure a rapid metabolism of purine nucleosides taken up from the medium. In contrast, *xapA* is expressed only if the inducer xanthosine is present in the growth medium. The xanthosine-induced activation of *xapA* expression is mediated by the regulatory protein XapR (Jorgensen, Dandanell, 1999). Apart of XapR, the following transcription factors control the expression of the genes of the salvage pathways: FNR (*nrdDG*), FUR (*nrdHIEF*), Fis (*nrdAB*), Nac (*codBA*), CRP (*hpt*), CRP, CytR (*udp*, *cdd*, *cytR*, *deoCABD*), DeoR (*deoCABD*), IHF (*hpt*). In addition to the transcription level, salvage pathway genes are regulated at the translation level. Expression of the *upp* gene of *E. coli*, which encodes the pyrimidine salvage enzyme uracil phosphoribosyltransferase, is negatively regulated by pyrimidine availability. The regulation occurs mainly by UTP-sensitive selection of alternative transcriptional start sites, which produces transcripts that differ in the ability to be productively elongated (Tu, Turnbough, 1997).

Application of the method of generalized Hill functions to modeling the molecular processes of salvage pathways can be exemplified by regulation of the activity of adenine phosphoribosyltransferase (APRT, coded by *apt* gene) in *E. coli*. The enzyme catalyzes a salvage reaction yielding AMP (Table 1). It is known that all acyclic nucleoside-5'-phosphates considerably inhibit APRT activity by competition for the substrate PRPP (Hochstadt-Ozer and Stadtman, 1971). An equation for the steady-state rate of the reaction is proposed:

$$V = \frac{k_{cat} \cdot e_0 \cdot \frac{S_1}{K_{m,S_1}} \cdot \frac{S_2}{K_{m,S_2}}}{\left(1+\frac{S_1}{K_{m,S_1}}\right) \cdot \left(1+\frac{S_2}{K_{m,S_2}}+\frac{P_1}{K_{i,P_1}}+\frac{P_2}{K_{i,P_2}}+\sum_{j=1}^{5}\frac{R_j}{k_{i,R_i,S_2}}+\sum_{j=6}^{10}\left(\frac{R_j}{k_{i,R_i,S_2}}\right)^2+\frac{R_{11}}{k_{i,R_{11},S_2}}\right)} \cdot \frac{1}{1+kl_{R_{12}} \cdot \frac{R_{12}}{k_{R_{12}}+R_{12}}}, \quad (1)$$

where e_0 is APRT concentration; S_1, S_2, P_1, P_2, R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10}, R_{11}, R_{12} are concentrations of low-molecular-weight substances AD, PRPP, PPI, AMP, ADP, dADP, ATP, dATP, dAMP, GTP, ITP, XTP, UTP, GDP, Mg^{2+}, and cAMP, respectively; k_{cat} is the catalytic constant; $K_{m,Si}$ are the Michaelis constants for corresponding substrates; $K_{i,Pi}$, constants of inhibition by corresponding products; $k_{i,Ri,S2}$, constants of inhibition by the corresponding regulator competing for the substrate PRPP; and kl_{R12}, k_{R12}, constants determining the efficiency of the effect of cAMP on the reaction rate.

Experimental data reported in (Hochstadt-Ozer and Stadtman, 1971) were used for testing the model of regulation of APRT activity. These data illustrate the effects of various low-molecular-weight substances (see comments on Eq. (1)) on APRT activity at various concentration combinations (Fig. 1).

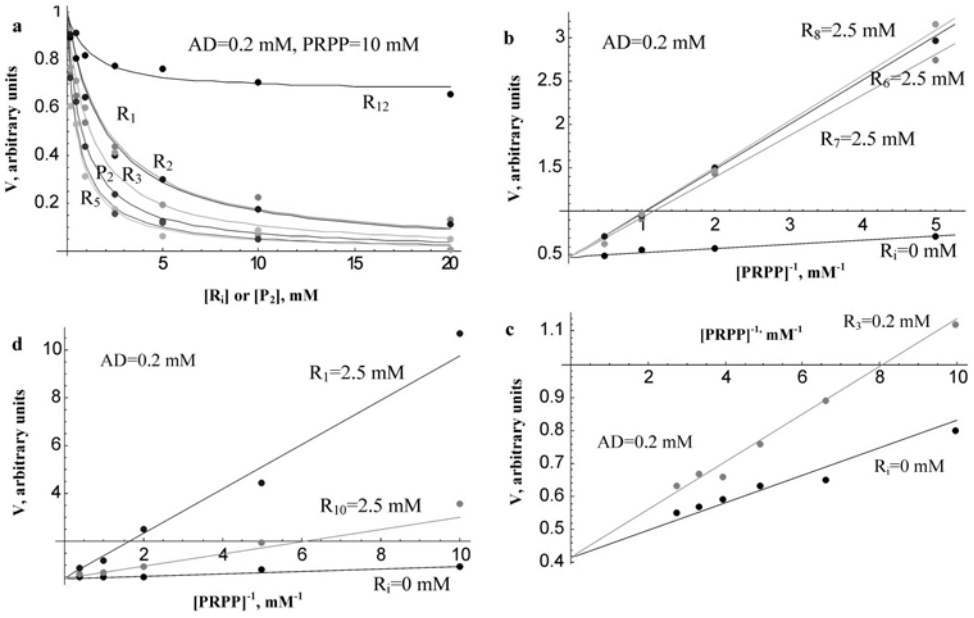

Figure 1. Effect of various regulators (for designations see text, comments on Eq. (1)) on the rate of the reaction catalyzed by APRT (*a*). Effect of PRPP of the rate of the reaction catalyzed by G6P1D at various concentrations of regulators Ri. (for each predicted curve $R_j = 0$ mM, at $j \neq i$) and AD = 0.2 mM (*b*, *c*, *d*). Dots indicate experimental values reported in (Hochstadt-Ozer, Stadtman, 1971). Curves are results of calculation according to Eq. (1) at the following parameter values: k_{cat} = 560 min^{-1}; $K_{m,S1}$ = 0.011 mM; $K_{m,S2}$ = 0.1 mM; $K_{i,P1}$ = 0.8 mM; $K_{i,P2}$ = 0.03 mM; $k_{i,R1,S2}$ = 0.13 mM; $k_{i,R2,S2}$ = 0.02 mM; $k_{i,R3,S2}$ = 0.27 mM; $k_{i,R4,S2}$ = 0.008 mM; $k_{i,R5,S2}$ = 0.0055 mM; $k_{i,R6,S2}$ = 0.79 mM; $k_{i,R7,S2}$ = 0.84 mM; $k_{i,R7,S2}$ = 2.0; k_{iR8S2} = 0.8 mM; $k_{i,R11,S2}$ = 10 mM; $k_{i,R12,S2}$ = 1.7 mM; kl_{R12} = 0.5; $k_{i,R9,S2}$ = 0.27 mM; and $k_{i,R10,S2}$ = 0.27 mM.

DISCUSSION

The reaction catalyzed by APRT is an example of multicomponent and complex regulation of a molecular system involving numerous structurally similar components. When this feature of the cellular system is taken into account, the connectivity of the graph representing the system increases dramatically. Molecular systems can also acquire this property because of a vast number of nonspecific interactions in the cell. Thus, the enzymatic system under discussion concerns a basic property of the cell. Consideration of such features in mathematical modeling of molecular systems is of paramount importance for proper description of actual molecular processes in a living cell.

Reconstruction of the gene network and development of mathematical models describing the efficiency of operation of enzymatic systems are essential for constructing a general kinetic model of salvage pathways. Such a model would allow predicting the progress of processes in the system, understanding their mechanisms, determining key links of the gene network, and analyzing effects of mutations on its operation. It will be an inextricable part of the "*in silico* cell" computer resource.

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