RECONSTRUCTION AND MATHEMATICAL MODELING OF THE GENE NETWORK CONTROLLING CYSTEINE BIOSYNTHESIS IN *ESCHERICHIA COLI*: REGULATION OF SERINE ACETYLTRANSFERASE ACTIVITY

***Mishchenko E.L.*[*1], *Lashin S.A.*[1], *Usuda Y.*[2], *Matsui K.*[2]**
[1] Institute of Cytology and Genetics, SB RAS, Novosibirsk, 630090, Russia; [2] Institute of Life Sciences, Ajinomoto, Co., Inc., Kawasaki, Japan
[*] Corresponding author: e-mail: elmish@bionet.nsc.ru

Key words: mathematical modeling, bioinformatics, cysteine biosynthesis, regulation, *Escherichia coli*

SUMMARY

Motivation: Development of an *in silico* cell as a computer resource for the modeling and analysis of intracellular processes is a topical problem of the systems biology.

Results: This work reconstructed the gene network of cysteine biosynthesis and degradation in *E. coli* cell. In the context of generalized Hill functions, the mathematical models describing the functioning efficiencies of enzymatic systems and expression regulation of the genes coding for enzymes and their subunits are constructed.

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

INTRODUCTION

The synthesis of L-cysteine from inorganic sulfur is the predominant mechanism by which reduced sulfur is incorporated into organic compounds; it produces significant quantities of L-cysteine only in plants and microorganisms, including *E. coli.* In this process, inorganic sulfate[5] is taken up and reduced to sulfide which is then incorporated into L-cysteine by a relatively simple two-step process requiring conversion of L-serine to O-acetyl-L-serine, which then reacts with sulfide. The reduction of sulfate requires its prior activation to a phosphosulfate mixed anhydride. This activation is achieved by the ATP sulfurylase-catalyzed reaction of sulfate with ATP to give adenosine 5′-phosphosulfate (APS) and PPi. Then APS kinase phosphorylates APS with another ATP to give PAPS which is then reduced by PAPS reductase to sulfite. The reduction of sulfite to sulfide is catalyzed by NADPH-sulfite reductase. Serine transacetylase catalyzes the acetylation of L-serine by acetyl-CoA to give O-acetyl-L-serine, the direct precursor of L-cysteine. The enzyme is feedback inhibited by L-cysteine and L-serine, thus providing kinetic regulation of this short branch of the pathway. Synthesis of L-cysteine from O-

[5] The abbreviations used are: SLF, Sulfate; APS, adenosine 5′-phosphosulfate; PPi, pyrophosphate; Pi phosphate; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PAP, 3′-phosphoadenosine 5′-phosphate; RTHIO, reduced thioredoxin; OTHIO, oxidated thioredoxin; ACSER, O-acetyl-L-serine; AC, acetic acid; ACCOA, acetyl-CoA; COA, CoA; PYR, pyruvate; ACC, acetylcysteine; CSA, L-cysteic acid; SAT, serine acetyltransferase.

acetyl-L-serine and sulfide is catalyzed by two distinct O-acetylserine(thiol)-lyase isozymes (Kredich, 1996).

METHODS AND ALGORITHMS

The gene network of regulation respiration was reconstructed with the GeneNet technology (Ananko *et al*., 2005). Mathematical models were constructed by the method of generalized Hill functions (Likhoshvai, Ratushny, 2006).

RESULTS AND DISCUSSION

The reconstructed gene network of Cysteine biosynthesis and degradation in *E. coli* cell is shown on the Fig. 1. Table 1 shows the number of components of the gene network of Cysteine biosynthesis and degradation.

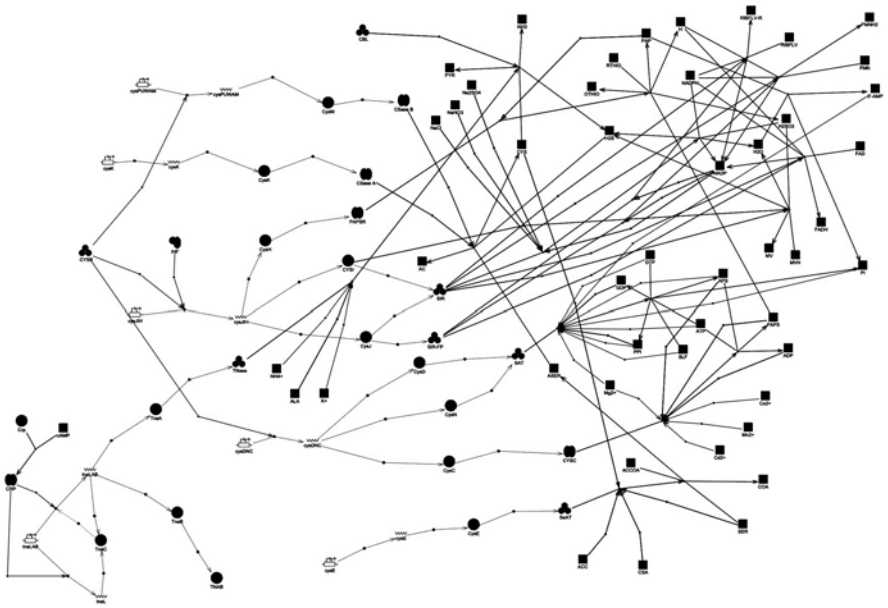

Figure 1. Cysteine biosynthesis and degradation gene network reconstruction in the GeneNet.

Table 1. List of the components of gene network of Cysteine biosynthesis and degradation

Item	Protein	RNA	Operon	Gene	Reaction	Substance	Literature source
Amount	27	7	6	15	100	48	112

Table 2 lists the enzymatic reactions involved in the gene network reconstruction, names of the enzymes catalyzing the corresponding reactions, and names of the genes encoding the corresponding enzymes. The mathematical models of enzymatic reactions (Table 2, column Reaction) and the models describing expression regulation of the genes encoding the corresponding enzymes and their subunits (Table 2, column Gene) were constructed using the method of generalized Hill functions. Table 3 shows the number of kinetic parameters for the enzymatic reactions and the regulatory processes (Michaelis constants, K_m; constants of catalytic activity, k_{cat}; constants of inhibition, K_i; constants of activation, K_a; the equilibrium constants of the dissociation, K_d; Hill coefficients, n^H) used

upon modeling of functioning efficiencies of enzymes. The set of other parameters of these models were determined by numerical experiments. It was demonstrated that the equilibrium and dynamic characteristics of the gene network in question calculated using the models developed fit the experimental data.

Table 2. Genes, enzymes and reactions of the gene network of Cysteine biosynthesis and degradation

Enzyme	Gene	Reaction
Sulfate adenylyltransferase	cysDN	SLF+ATP+GTP → PPi+APS+GDP+Pi
Adenylylsulfate kinase	cysC	APS +ATP →ADP +PAPS
3'-Phospho-adenylylsulfate reductase	cysH	PAPS +RTHIO →OTHIO+H2SO3+PAP
Sulfite reductase	cys IJ	H2SO3 +3NADPH↔H2S+3NADP
Serine acetyltransferase	cysE	SER+ACCOA↔COA+ACSER
O-Acetylserine (thiol)-lyase A	cysK	ACSER +H2S→AC+CYS
O-Acetylserine (thiol)-lyase B	cysM	ACSER +H2S→AC+CYS
Tryptophanase	tnaA	CYS →PYR+NH3+H2S
L-Cysteine desulfhydrase		CYS →PYR+NH3+H2S

Table 3. Kinetic parameters for the enzymatic reactions and the regulatory processes involved in the gene network reconstruction

	Kinetic parameters						
	Michaelis-Menten constant K_m	Constant of catalytic activity k_{cat}	Maximal velocity V_{max}	Dissociation constant K_d	Hill coefficient h	Constant of activation K_{act}	Constant of inhibition K_i
Amount	49	12	1	25	6	4	16

Let us consider the model of enzyme serine acetyltransferase (Kredich, Tomkins, 1966) activity regulation as an example of modeling method. In this reaction, L-cysteine is competitive inhibitor, L-serine, L-cysteic acid and acetylcysteine are also inhibit the enzymatic activity at the concentrations of 1–4 mM.

A model for the steady-state rate of the reaction is proposed (where e_0 is the concentration of the enzyme serine acetyltransferase; SER, ACCOA, ACSER, COA, ACC, CYS, CSA are concentrations of the corresponding low-molecular-weight substances; k_f, the catalytic constant of direct reaction, k_r, the catalytic constant reverse reaction; $K_{m,SER}$, $K_{m,ACCOA}$, $K_{m,ACSER}$, $K_{m,COA}$, Michaelis constants for corresponding substrates and products; $K_{i,ACC}$, $K_{i,CYS}$, corresponding inhibition constants; k_{SER}, $l_{i,SER}$, constants of substrate inhibition efficiency (SER), h_{SER} constant determining the nonlinearity of the effect of SER on the reaction rate; k_{CSA}, constant of the efficiency of the effect of CSA on the reaction rate).

$$V = \frac{e_0 \cdot \left(\mathrm{k_f} \cdot \frac{\mathrm{SER}}{\mathrm{K_{m,SER}}} \cdot \frac{\mathrm{ACCOA}}{\mathrm{K_{m,ACCOA}}} - \mathrm{k_r} \cdot \frac{\mathrm{ACSER}}{\mathrm{K_{m,ACSER}}} \cdot \frac{\mathrm{COA}}{\mathrm{K_{m,COA}}} \right)}{\left(1 + \frac{\mathrm{SER}}{\mathrm{K_{m,SER}}} + \frac{\mathrm{ACSER}}{\mathrm{K_{m,ACSER}}} + \frac{\mathrm{ACC}}{\mathrm{K_{i,ACC}}} \right) \left(1 + \frac{\mathrm{ACCOA}}{\mathrm{K_{m,ACCOA}}} + \frac{\mathrm{COA}}{\mathrm{K_{m,COA}}} + \frac{\mathrm{CYS}}{\mathrm{K_{i,CYS}}} \right)} \cdot \frac{1 + \left(\frac{\mathrm{SER}}{\mathrm{k_{SER}}} \right)^{h_{SER}}}{1 + \left(l_{i,SER} \cdot \frac{\mathrm{SER}}{\mathrm{k_{SER}}} \right)^{h_{SER}}} \cdot \frac{1}{1 + \left(\frac{CSA}{k_{CSA}} \right)}$$

The experimental data (Kredich *et al.*, 1966) were used for model testing. These data illustrate the effects of the substrates SER and ACCOA on SAT activity with various concentrations of CYS and COA (Fig. 2).

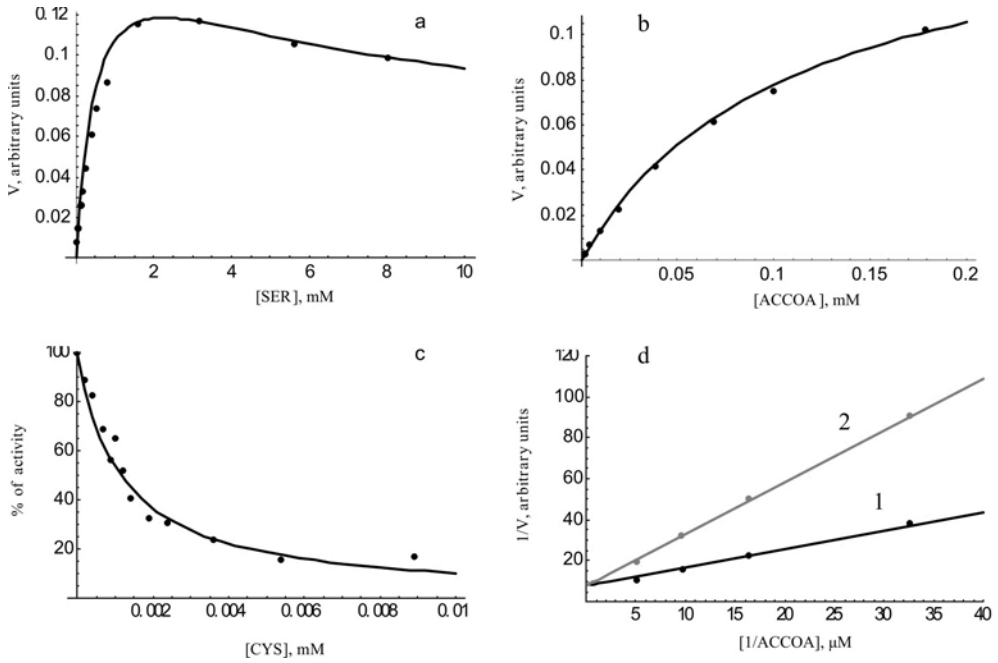

Figure 2. Effect of SER on the rate of the reaction catalyzed by SAT (*a*); effect of ACCOA on the rate of the reaction catalyzed by SAT (*b*) ; effect of CYS on the rate of the reaction catalyzed by SAT (*c*, *d*). The enzyme activity was measured (*a*) with 0.1 mM ACCOA, SAT concentration equaling 0.4 mM; (*b*) with 1 mM SER, SAT concentration equaling 0.29 mM; (*c*) with 1 mM SER, 0.1 mM ACCOA, SAT concentration equaling 0.4 mM; (*d*) 1 mM SER, at CYS concentrations: 1, null; 2, 1.1 μM. Dots indicate experimental data (Kredich *et al*., 1966), and curves are the results of simulation according to model (1) with the following parameters: $K_{m,SER}$ = 0.56 mM, $K_{m,ACCOA}$ = 0.11 mM, $K_{i,ACC}$ = 1 mM, $K_{i,CYS}$ = 0.6 μM, k_{SER} = 18 mM, h_{SER} = 1.1, $l_{i,SER}$ = 3.5, k_f = 10 u/s k_r = 1 u/s.

The model parameters are estimated according to the correspondence of experimental data. The proposed mathematical model and the other eight models of enzymatic reactions developed in the framework of the study are the components of more generalized model of the cysteine biosynthesis in the *E. coli* cell. On the next stage we plan to describe genetic regulation of expression of enzymes and their subunits. Then we will be able to analyze functioning of the cysteine biosynthesis pathway taking into account genetic processes. Furthermore, the developed models will be the significant component of the computer resource – an *in silico* cell.

ACKNOWLEDGEMENTS

The authors are grateful to Vitaly Likhoshvai for valuable discussions and to Irina Lokhova for bibliographical support. This work was supported in part by the Russian Government (Contract No. 02.467.11.1005), by Siberian Branch of the Russian Academy of Sciences (the project “Evolution of molecular-genetical systems: computational analysis and simulation” and integration projects Nos. 24 and 115), and by the Federal Agency of Science and Innovation (innovation project No. IT-CP.5/001).

REFERENCES

Ananko E.A., Podkolodny N.L., Stepanenko I.L., Podkolodnaya O.A., Rasskazov D.A., Miginsky D.S., Likhoshvai V.A., Ratushny A.V., Podkolodnaya N.N., Kolchanov N.A. (2005) GeneNet in 2005. *Nucl. Acids Res.*, **33,** D425–D427.

Kredich N.M. (1996) Biosynthesis of Cysteine. In Neidhardt F.C. (ed.), *Escherichia coli and Salmonella typhimurium*. Washington, 514–527.

Kredich N.M., Tomkins G.M. (1966) The enzymic synthesis of L-cysteine in Escherichia coli and Salmonella typhimurium. *J. Biol. Chem*., **241**, 4955–4965.

Likhoshvai V.A., Ratushny A.V. (2006) *In silico* cell I. Hierarchical approach and generalized hill functions in modeling enzymatic reactions and gene expression regulation. *This issue.*