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Use of the Flux Model of Amino Acid Metabolism of *Escherichia coli*

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Abstract—A program implementing a flux model of *Escherichia coli* metabolism was used to analyze the effects of the addition of amino acids (tryptophan, tyrosine, phenylalanine, leucine, isoleucine, valine, histidine, lysine, threonine, cysteine, methionine, arginine, proline) to minimal medium or media lacking nitrogen, carbon, or both. The overall response of the metabolic system to the addition of various amino acids to the minimal medium is similar. Glycolysis and the synthesis of pyruvate with its subsequent degradation to acetate via acetyl-CoA become more efficient, whereas the fluxes through the pentose phosphate pathway and the TCA cycle decrease. If amino acids are used as the sole source of carbon, nitrogen, or both, the changes in the flux distribution are determined mainly by the carbon limitation. The phosphoenolpyruvate to glucose-6-phosphate flux increases; the flux through the pentose phosphate path is directed towards ribulose-5-phosphate. Other changes are determined by the compounds that are the primary products of catabolism of the added amino acid.

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Functioning of an organism is a complex process in which the synchronized operation of biological systems is achieved via multi-stage interactions between molecular components of a cell. A variety of approaches have been developed to analyze the genome of an organism. One such approach connects the characteristics of the DNA sequence with the properties of intracellular systems based on fundamental physicochemical laws. An example of such strategy is flux modeling, which aims at a description of all metabolic fluxes characterizing intensities of chemical reactions in a cell at equilibrium.

A flux model is constructed in two steps. In the first step, the gene complement of a genome is used to determine a number of corresponding chemical reactions. In the second step, compositions of the biomass and the growth medium are specified. Finally, the fluxes are calculated by maximizing the biomass growth for various media and sets of chemical reactions.

The flux model for *Escherichia coli* is the most complete one, and it has produced several important results.

A mutant cell is believed to change its fluxes in order to compensate for the absence of a particular enzyme and to reach the initial growth rate [1]. The flux model allows one to compute the fluxes corresponding to varying inhibited genes. For the case of *E. coli*, flux changes have been computed for sequential intimation of the central metabolism genes. If the parameters of gene repression or activation are known, they can be used as additional constraints for the flux modeling.

Seven enzymes of *E. coli* central metabolism are crucial for aerobic cell growth on glucose minimum [2, 3]. For anaerobic growth on glucose minimal medium, 15 enzymes seem to be most important. The total of 49 basic enzymes of the central metabolism were studied [2, 3]. The relatively small number of enzymes required for the aerobic and anaerobic cell growth demonstrates that the *E. coli* metabolic system has few requirements and is highly robust.

Several studies have modeled the effect of different media on flux models [4, 5]. The results obtained for the glucose medium are consistent with experimental observations, while those for the nitrogen limiting medium disagree with the experimental data. The success of the flux modeling of the glucose media may reflect the fact that *E.*

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coli normally gets sufficient nitrogen but has limited carbon sources [6].

The goal of this study was to examine the changes of the metabolic fluxes in response to addition of amino acids to the minimal medium, or to medium lacking nitrogen, carbon, or both nitrogen and carbon. Five groups of amino acids were considered: 1) aromatic (tryptophan, tyrosine, phenylalanine); 2) branched (leucine, isoleucine, valine); 3) histidine, lysine, threonine; 4) sulfur-bearing (cysteine, methionine); 5) arginine, proline.

METHODS OF INVESTIGATION

The algorithm was as follows. All metabolic processes are constrained by the mass balance limitations. Calculation of the cell system capabilities based on these constraints is known as flux balance analysis [7]. For a metabolic system, mass balance can be written in the form of a matrix equation:

$$S \cdot \mathbf{V} = 0,$$

where S is a stoichiometric matrix. The matrix size is $m \times n$, where m is the number of metabolites, and n is the number of chemical reactions in the system. The vector $\mathbf{V} = v_i$, where $i = 1, 2, ..., n$, describes all the fluxes of a metabolic system via their intensities.

The number of fluxes in this model (n) exceeds the number of mass balance constraints (i.e., the number of metabolites, m) ($m < n$). This problem is, therefore, underdetermined, and it has an infinite number of solutions corresponding to different possible flux distributions.

To reduce the number of solutions, an additional constraint is used:

$$\alpha_i \le v_i \le \beta_i,$$

where α_i and β_i are constants. The α_i and β_i parameters may reflect the possible reversibility of a chemical reaction (the reverse reaction corresponds to the negative sign before α_i or β_i), or to define the flux values. With the above equations, the solution search is performed by maximizing a criterion function (that describes, for example, the biomass growth). Thus, the medium is defined by the choice of the v_i values in the form of linear inequalities ($\alpha_i \le v_i \le \beta_i$). For example, the minimal medium in our computations is defined by the presence of 20 mole fractions of oxygen ($\alpha_{O2} = -20$), 10 mole fractions of glucose ($\alpha_{glc} = -10$), and unlimited amount of hydrogen, sodium, potassium, iron, sulfate, phosphate, ammonium, water, and carbonic gas ($\alpha_j = -10{,}000$). The compounds for which a corresponding excretory chemical reaction exists ($\beta_j = 10{,}000$) can be removed from a cell in any amount.

The criterion function is defined as follows:

$$Z = \Sigma c_i v_i = <\mathbf{c}\cdot\mathbf{V}>,$$

where vector $\mathbf{c}$ is a unit vector (vector length is n) directed towards biomass growth. The growth flux can be determined based on the biosynthetic needs of a cell:

$$\Sigma d_m \cdot X_m > \text{biomass},$$

where d_m is the fraction of compound X_m in the cell biomass. Therefore, the growth flux can be modeled as a single process transforming all biosynthetic precursors into biomass. The parameters of the biomass equation are determined from the experimental data.

This problem can thus be regarded as a problem of linear programming. It is solved based on the assumption that a cell tends to increase its biomass for faster proliferation.

The software used in this study has been developed by the laboratory (Mathematical Methods and Models in Bioinformatics) of the Institute for Information Transmission Problems of the Russian Academy of Sciences. The data on the software can be obtained by E-mail at rubanov@iitp.ru.

The data on *E. coli* metabolism were obtained from http://gcrg.ucsd.edu/supplementary_data/DeletionAnalysis/main.htm. The maps of metabolic pathways were downloaded from the MetaCyc database (http://www.metacyc.com/).

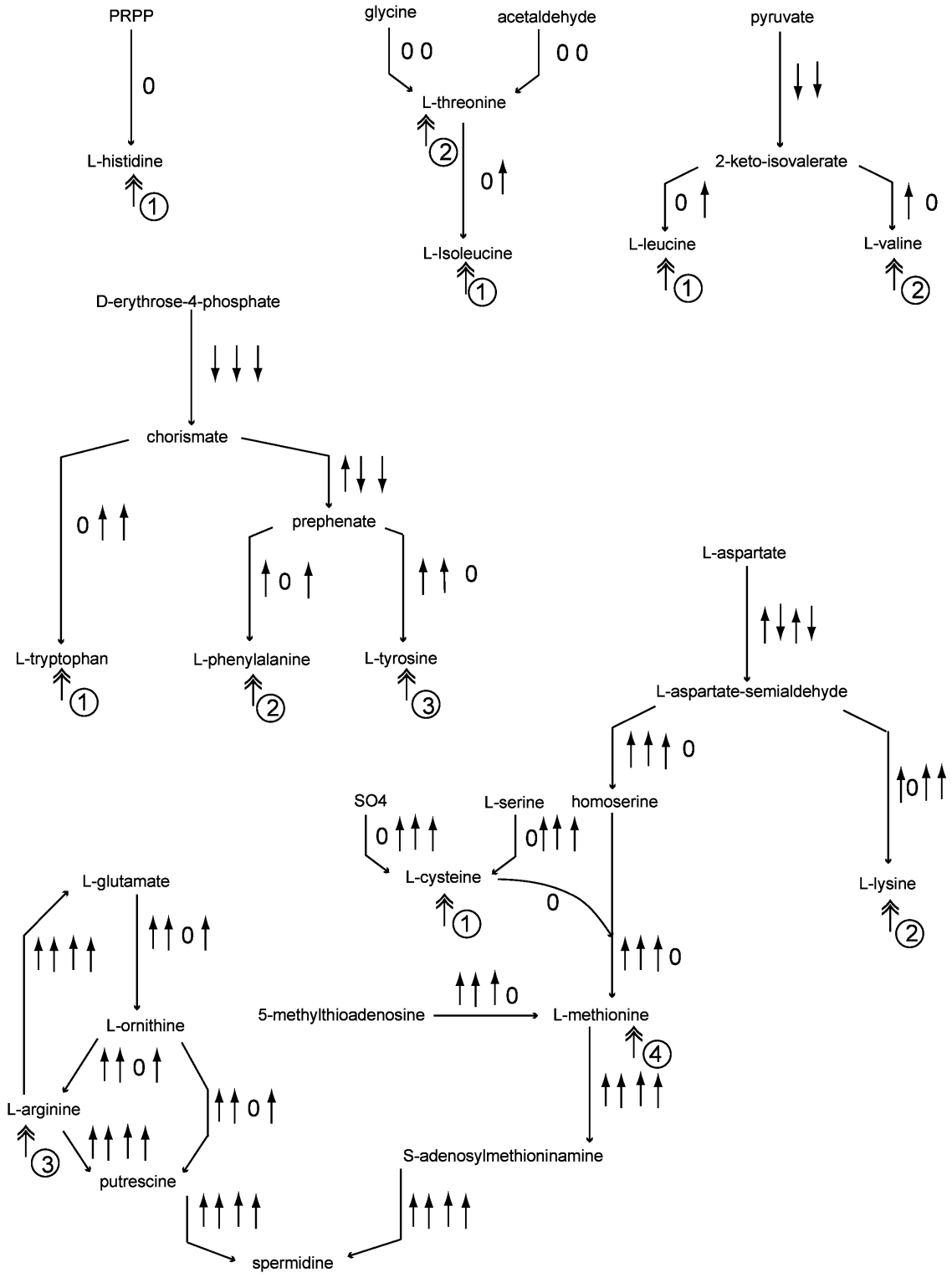
RESULTS AND DISCUSSION

Ideas about the structure of metabolic fluxes were confirmed and some non-trivial observations were developed.

When a flux is just a linear sequence of chemical reactions, all reactions should change consistently. If a flux is split into two smaller fluxes, i.e., a metabolite is generated by one reaction and utilized by two exit reactions, the total value of the entrance flux must be equal to the sum of the two exit fluxes.

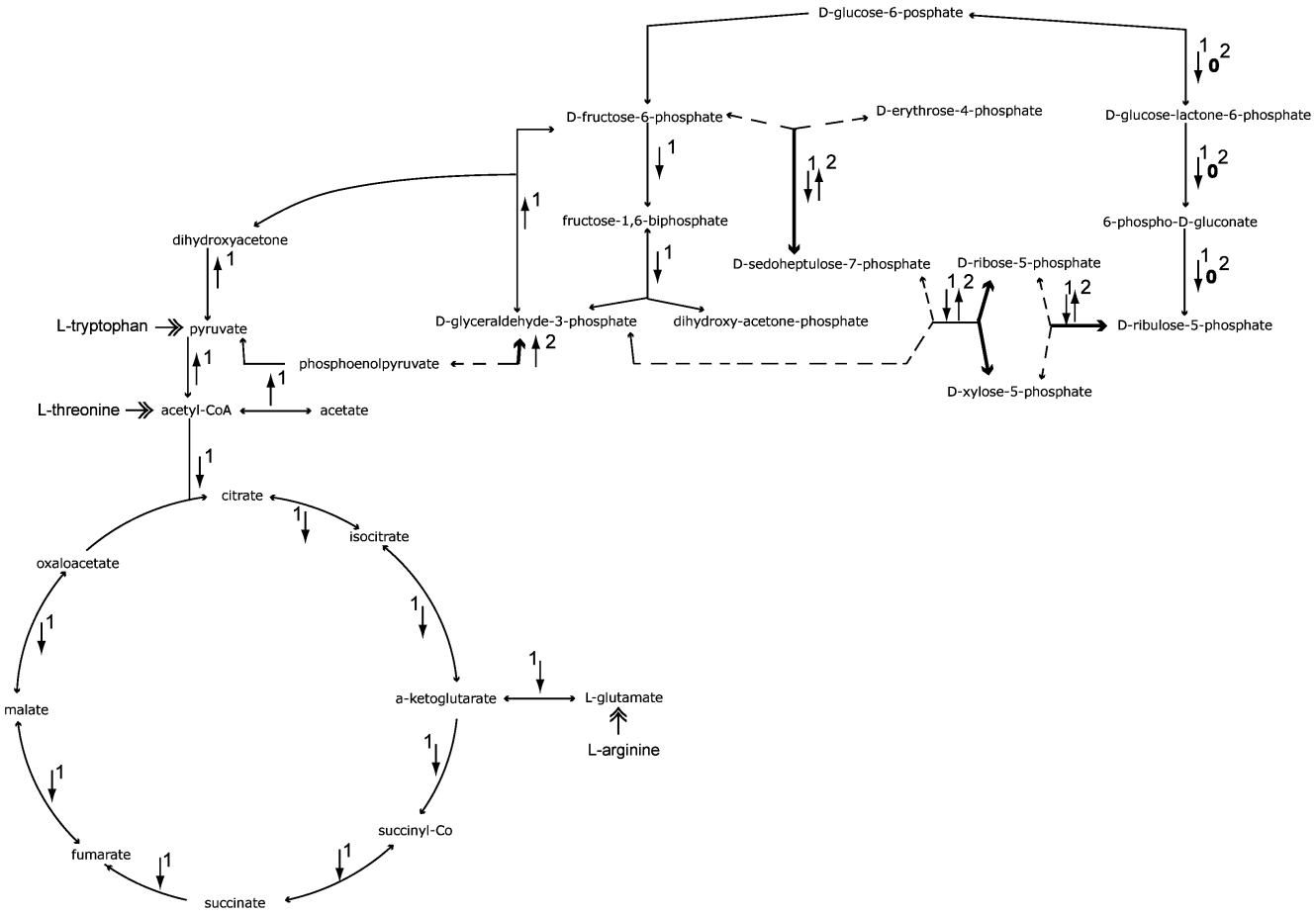
A cell prefers to import a substance rather than synthesize it because the decrease in the intensities of the synthesis fluxes leads to the release of consumable metabolites. Excessive amount of a metabolite is removed from a cell through its membrane, sometimes after being chemically transformed, by transport reactions.

The production of biomass increases if the minimal medium is enriched by additional compounds, which leads to the nonspecific growth of all fluxes, except for those corresponding to the synthesis of the compound. An amino acid may be used as a substrate for the synthesis of basic metabolites if it is present in the cell in significant excess, and there are corresponding catabolic path-



Changes in metabolic fluxes for the synthesis and catabolism of amino acids in response to addition of amino acids to minimal medium. Five graphs illustrate the changes in flux intensity associated with metabolism of the studied amino acids. Many intermediate stages of synthesis are not shown. The most important initial and intermediate metabolites and the resultant amino acids are shown as graph nodes. Changes in fluxes associated with addition of an amino acid are indicated on the right side of a graph. "0" corresponds to the case when the flux intensity tends to zero upon addition of the amino acid. A single upward arrow indicates increase in the flux intensity; a single downward arrow indicates decrease in the flux intensity. Double arrows point to amino acids added to the system. The changes in metabolism resulting from addition of amino acids are shown by sequences of numbered double arrows

Scheme 1



Changes in the central metabolism of *E. coli* in response to addition of amino acids to minimal medium (1) and to medium lacking carbon sources (2). A single upward arrow indicates an increase in flux intensity; a single downward arrow indicates a decrease in flux intensity. Marked branches correspond to reversible reactions that change their direction depending on the medium. Flux directions are shown in dotted lines for minimal medium, and in bold lines for media lacking carbon. Double arrows indicate "entry points" of the amino acid catabolism

Scheme 2

ways. For example, there are two alternative pathways for the synthesis of putrescine, one starting with ornithine and the other with arginine. Thus, putrescine is synthesized from ornithine in case of arginine deficiency and from excessive arginine in the opposite way. Scheme 1 presents a schematic sketch illustrating these results.

The addition of amino acids to the minimal medium affects flux intensities of glycolysis, glyconeogenesis, citrate cycle, pentose phosphate pathway, and metabolism of pyruvate and glutamate (Scheme 2). In the presence of an amino acid, a cell partly synthesizes D-glyceraldehyde-3-phosphate directly from D-fructose-6-phosphate bypassing the intermediate fructose-1,6-bisphosphate. Dihydroxyacetone is a byproduct of this direct catabolism pathway. The intensity of the dihydroxyacetone catabolism increases. The intensity of flux through the pentose phosphate pathway decreases. Apparently, this is a consequence of decreased NADPH consumption.

The amount of synthesized pyruvate increases. Intensities of degenerative fluxes from pyruvate to acetyl coenzyme A also increase. At the same time, acetyl coenzyme A converts to acetate at a faster rate. The intensities of transport reactions for acetate also increase. The degradation of pyruvate to acetate thus becomes more intense.

The intensity of the citrate cycle decreases. When amino acids are added to the medium, a cell does not need to synthesize some substances anymore, which results in smaller amounts of produced NADH. For this reason, the intensity of the citrate cycle (which is one of the most effective sources of energy in the cell) decreases. The intensities of the reactions directly related to the citrate cycle also decrease.

The above results suggest that the cell can (at least partially) shift from a more advantageous but relatively long energy production pathway (the citrate cycle) to a

less advantageous but shorter one (degradation of pyruvate), since the amount of energy needed for the growth decreases upon addition of amino acids.

Both the transformation rate of glutamate to α-ketoglutarate and the carbonic acid export become less intensive. The drop in the carbonic acid transport reaction may be a consequence of a lower efficiency of the citrate cycle and the pentose phosphate pathway.

When the medium lacks glucose, nitrogen, or both, the use of an amino acid as a substrate is possible only if a catabolic pathway for the amino acid exists. Our model has three such pathways for arginine, tryptophan, and threonine. The model can be optimized only if there is an excess of an amino acid in the medium (in comparison with the minimum amount of amino acid needed for cell growth). Part of this minimum amount of amino acid goes as it is in the biomass and the rest must supply the whole cell metabolism. As a result, the flux intensities for the synthesis of this amino acid are always zero.

An amino acid may be a source of carbon or nitrogen depending on which of them is lacking in the medium. The following basic metabolites are produced if amino acids serve as a source of carbon: glutamate from arginine, acetyl coenzyme A from threonine, and pyruvate from tryptophan. Ammonium ions are synthesized if the amino acid is a source of nitrogen.

If the medium lacks glucose, the changes in the intensities of the glycolysis and pentose phosphate pathways are similar regardless which amino acid is added: the pathway from phosphoenolpyruvate to glucose-6-phosphate increases, and the pentose phosphate pathway is directed towards ribulose-5-phosphate. The changes in the fluxes of the citrate cycle and the metabolism of pyruvate and glutamate depend on what basic metabolite is produced by catabolizing the amino acid.

The initial biomass growth corresponding to ten parts of glucose is achieved very slowly in successive calculations with the growth of amino acid mole fraction. The tryptophan catabolic pathway seems to be the most efficient one, while the catabolism of arginine is the least efficient.

In the nitrogen lacking media, the fluxes change in order to compensate for the lack of ammonium ions, if the amount of added amino acid is minor. If more of the amino acid is added, the nitrogen excess is extruded from the cell. At the same time, the substances replacing glucose are synthesized, and this case is thus reduced to the one described above. This results in faster increase in the biomass. This process is limited only by the amount of oxygen in the medium. If there is a significant excess of amino acid in the medium, and nitrogen is lacking, the flux distribution in the cell is identical to the one corresponding to the medium lacking glucose, since the catabolic fluxes of amino acids compensate the shortage of nitrogen and the amount of glucose in the medium is negligible in comparison with the amount of the amino acid.

In the case of insufficient amount of oxygen, the cell partly switches from aerobic to anaerobic metabolism in order to achieve the maximum biomass. Arginine is the most effective substrate with the nitrogen-lacking medium. The shortage of ammonium ions is compensated faster by adding the amino acid, which is further used as an additional substrate for biomass growth. The growth is determined by the fluxes compensating the shortage of glucose in the medium lacking both nitrogen and carbon sources.

The above results demonstrate that a metabolic system responds similarly to the addition of different amino acids into the minimal media.

In the case when amino acids are the only source of carbon in the cell, some fluxes change in a similar way, while others behave differently depending on what basic metabolite is produced by catabolizing of the added amino acid, i.e. on the "point of entrance" of the catabolic pathway in the central metabolism.

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