MODELING OF NITRITE UTILIZATION IN THE *E.COLI* CELL: FLUX ANALYSIS

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E.coli uses different acceptors in electron transport chain under the anaerobic respiration including nitrite. Given the high toxicity of nitrite for a cell, regulation of genes expression included in the nitrite-associated electron transport chain, is closely related to genes of nitrite metabolism and its transport into as well as out the cell. Components of the system are nitrite reductases NrfA, NirB and the transport protein NirC. So far the specific data on the relationship between the nitrite respiratory, transport and utilization systems in the cell is absent. The current data on regulatory mechanisms of *nrfA, nirB* and *nirC* genes expression [1] allows us to develop a mathematical model of nitrite utilization in the *E.coli* cell and to study a role of NirB and NrfA reductases as well as NirC transport protein in the regulation of intracellular nitrite' content. The model reproduces glucose-limiting conditions of *E.coli* cells continuous cultivation in the chemostat. These conditions provide a permanent growth rate and density of the culture. The model describing these conditions is presented an autonomous system of equations:

$$\begin{cases} u' = k_{flow}s + CV_{NirC,out}(u,w) - C\left(V_{Nrf}(u) + V_{NirC,in}(u)\right) - k_{flow}u, \\ w' = V_{NirC,in}(u) - V_{NirC,out}(u,w) - V_{NirB}(u,w) - k_{flow}w. \end{cases}$$

where, u – the nitrite concentration in the chemostat, w – the same in the cell; k_{flow} – the rate constant of substances cycling in the chemostat; C – the relative part of the culture's volume in the chemostat; s – the nitrite concentration, which is reached in the empty chemostat; $V_{Nrf}(u)$, $V_{NirB}(w)$ – the utilization rates of extra- and intracellular nitrite by the NrfA and NirB reductases, $V_{NirC,in}(u)$, $V_{NirC,out}(u,w)$ – the rates of nitrite transport by NirC protein in/out the cell. The rate equations for each process approximating the kinetic data [1, 3] are based on the method of generalized functions Hill [2].

It has shown that the model adapted to the experimental data on dynamics of the *nrf* and *nir* operons expression [1] describes experimentally observed dynamics of the nitrite

accumulation in the chemostat at high concentrations of added nitrite (>2мМ) [3]. In the concentration area the permanent intracellular nitrite concentration which does not depend on its concentration in the growth environment is maintained in accordance to the model.

To describe the kinetics of nitrite accumulation in the chemostat at low concentrations of added nitrite (≤1мМ) the supplementary hypothesis about the existence of more high level of NrfA enzymatic activity than it observes in the genetic studies is required to be included in the model. There are model calculations of nitrite utilization and transport fluxes in as well as out the cell under abovementioned suggestions relatively the NrfA activity and maximal import rate of nitrite in the cell that equals 0.65 mM/sec (fig. 1). The rate value is close to measured NirC protein activity under the respiration in 20 mM nitrate [4]. It can be seen that the nitrite flux out the cell practically absents under these parameters of the NirC activity, but the dynamics of NirB activity qualitatively agrees with the experiment [1].

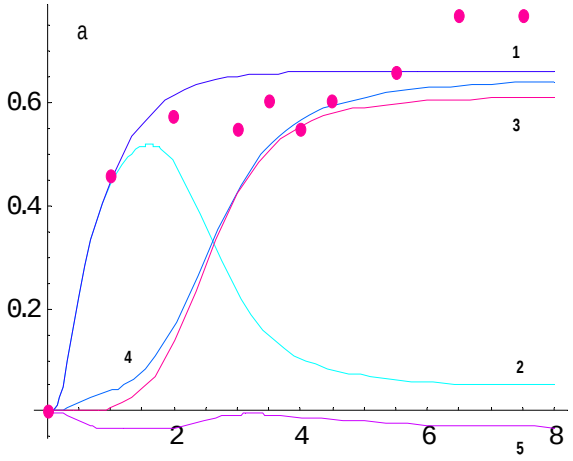

Fig.1 The rate of nitrite utilization by the NrfA and NirB reductases and the rate of its transport in/out the cell by the NirC protein. Curve 1 – approximation of the experimental points [3] by $1.2(1-\exp(-0.6u))/(1+\exp(-0.6u))$ function; curve 2 – the rate of nitrite utilization by NrfA reductase calculated by the model; curve 3 – the rate of intracellular nitrite consumption calculated as the difference between curves 1 and 2. Curve 4 – the rate of nitrite transport in the cell calculated by the model; curve 5 – the rate of nitrite transport out the cell calculated as the difference between curves 4 and 3. X-axis – the concentration of the added nitrite (мМ), Y-axis – the rate of nitrite consumption (мМ/sec).

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