INFLUENCE OF PEP GLYCOLYTIC PRECURSOR ON TRYPTOPHAN SYNTHESIS DYNAMICS IN *E. COLI* CELLS

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Average concentrations of glycolytic intermediates involved in the tryptophan (TRP) synthesis are of major importance in regulating and modulating the TRP synthesis. It is well known that oscillations of glycolytic intermediates often occur in living cells, according to the environment conditions and characteristics of the ATP-recovery system. As the TRP synthesis is connected to glycolysis through the PEP (phosphoenolpyruvate) node, the study of the major role played by the PEP glycolytic intermediate for the tryptophan (TRP) oscillatory metabolic synthesis is of high interest in industrial biosynthesis. Based on a reduced kinetic model for TRP synthesis from literature, this paper performs an in-silico analysis of the way by which the PEP level involved in the oscillatory glycolysis influences the TRP synthesis in E. coli bacteria. The analysis allows further TRP synthesis optimization.

Keywords: dynamic models; glycolysis; tryptophan synthesis; *Escherichia coli*; phosphoenolpyruvate

Abbreviations and notations [5]

13DPG, PGP	1,3-diphosphoglycerate	Glc, GLC	glucose
2PG	2-phosphoglycerate	GLCex, GLC(ex)	Glucose in the environment
3PG	3-phosphoglycerate	GLN	glutamine
AC	acetate	GRC	genetic regulatory circuits
AA	amino-acid	H	Hydrogen radical
ACCOA	acetyl-coenzyme A	HK-ase	hexokinase
AK-ase	adenylate kinase	LAC	lactate
AMDTP	adenosin-(mono)(di)(tri)phosphate lump	MAL	malate
ADP	adenosin-diphosphate	mM	Milli-molar
AMP	adenosin-monophosphate	MRNA	tryptophan mRNA during its encoding gene dynamic transcription, and translation
ATP	adenosin-triphosphate	NAD(P)H	nicotinamide adenine dinucleotide (phosphate) reduced
ATP-ase	ATP monophosphatase	O	TRP active gene
CCM	central carbon metabolism	OR	the complex between O and R

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			(aporepressor of the TRP gene)
CIT	citrate	OT	total TRP operon
CHR	chorismat	P, Pi	Phosphoric acid
DAHP	3-Deoxy-D-arabino-heptulosonic acid 7-phosphate	PEP	phosphoenolpyruvate
DHAP	dihydroxyacetonephosphate	PFK-ase	phosphofructokinase
DW	dry-weight	PK-ase	pyruvate kinase
E	enzyme anthranilate synthase	Phe	Phenylalanine
ETOH	ethanol	PPP	pentose-phosphate pathway
F6P	fructose-6-phosphate	PPH	prephenate
FAD	Flavin adenine dinucleotide	PTS	phosphotransferase, or phosphoenolpyruvate: glucose phosphotransferase system
FADH	semiquinone form of the reduced FAD	PYR	pyruvate
FADH2	hydroquinone form of the reduced FAD	QSS	quasi-steady-state
FDP	fructose-1,6-biphosphate	R	aporepressor of the TRP gene
FOR	formate	SUCC, SUC	succinate
G3P, GAP	glyceraldehyde-3-phosphate	TCA	tricarboxylic acid cycle
G6P	glucose-6-phosphate	TF	transcription factors
GKase	glucokinase	T, TRP	tryptophan
GERM	gene expression regulatory module	Tyr	Tyrosine
		X5P	Xylulose 5-phosphate
c_j	species *j* concentration	Indices	O =initial; syn= synthesis
D	cell content dilution rate	Index s	Stationary (quasi-steady-state)
k_j , K_j	rate constants	y_{trp}	stoichiometric coeff.
t , t_C	Time, Cell cycle	Super-script	n = reaction order

1. Introduction

"Autonomous oscillations of the glycolytic intermediates' concentrations reflect the dynamics of control and regulation of this major catabolic pathway, and the phenomenon has been reported in a broad range of cell types" [1]. "The study of glycolytic oscillations might therefore prove crucial for general understanding of the cell metabolism regulation and the connections among different parts of metabolism" [2]. "The key question in this context is the mechanism of the oscillations but, despite much work over the last 40 years, it remains unsettled" [1,2].

"Besides, glycolysis, together with the phosphotransferase (PTS)-system for glucose transport into the cell, the pentose-phosphate pathway (PPP), and the tricarboxylic acid cycle (TCA) characterize the central carbon metabolism (CCM)" [19].

Modelling bacteria CCM is a subject of great interest, allowing *in silico* design of modified cells with desirable characteristics of various applications in the biosynthesis industry, civil engineering, and other fields [18]. The interest for such a subject is even higher as long as most of the glycolysis intermediates are starting nodes for the internal production of lot of cell metabolites (e.g. amino-acids, SUCC, CIT). Valuable models are presented in the literature in this subject [3-6].

This paper is focused on simulation the tryptophan (TRP) synthesis in connection to its key-precursor, that is the PEP (phosphoenolpyruvate), which is one of the glycolysis key-node. "TRP is an aromatic non-polar α-amino-acid essential in humans, that is used in the cell biosynthesis of proteins, being also a precursor to the neuro-transmitter serotonin, of the melatonin hormone, and of vitamin PP" [7]. The paper subject is of high interest, because TRP is a metabolite of high practical importance, being important maximization of its production. This is why, intense efforts have been invested not only to understand its synthesis regulation mechanism in various micro-organisms, to construct an adequate dynamic model of its oscillatory synthesis, but also to determine conditions leading to speed-up its production. In the present paper, the TRP synthesis dynamics is studied for various PEP concentrations, in a broad range of average PEP levels covering the high dynamics of the glycolytic oscillations determined by external (GLC level), and internal factors (such as efficiency of ATP recovery system) [3-6].

2. The kinetic model of TRP synthesis in *E. coli* prokaryotic bacteria

"The TRP synthesis regulation being very complex, a significant number of simplified kinetic models with lumped terms (species/reactions) have been proposed in the literature" [3-6]. As reviewed by several authors, "oscillations in the TRP synthesis are produced due to the concomitant activation and high order repression of the TRP-operon expression, together with a nonlinear demand for end product, making its expresses to be cyclic. The cell growth and dilution rates (related to the cell cycle, and the liquid residence-time in the bioreactor) have also a strong influence on the TRP system stability" [4-6].

A simplified TRP synthesis pathway is displayed in Fig. 1 based on studies of [8-10]. Bhartiya et al. [11] proposed a simplified kinetic model for the TRP synthesis given in Table 1. This TRP model [11] adopted in this study (Table 1) suffered two modifications to match with the Bhartiya [11] experimental data, as suggested by Maria [4]: I) "The rate constant k_4 was re-estimated in order to fit the experimental curves of OR, mRNA, T, E species" of [11], (given in Fig. 2 for [PEP]s = 1 mM case), by using a classic estimator [12], and II) To be connected to the glycolysis pathway (as displayed in Fig. 1),.

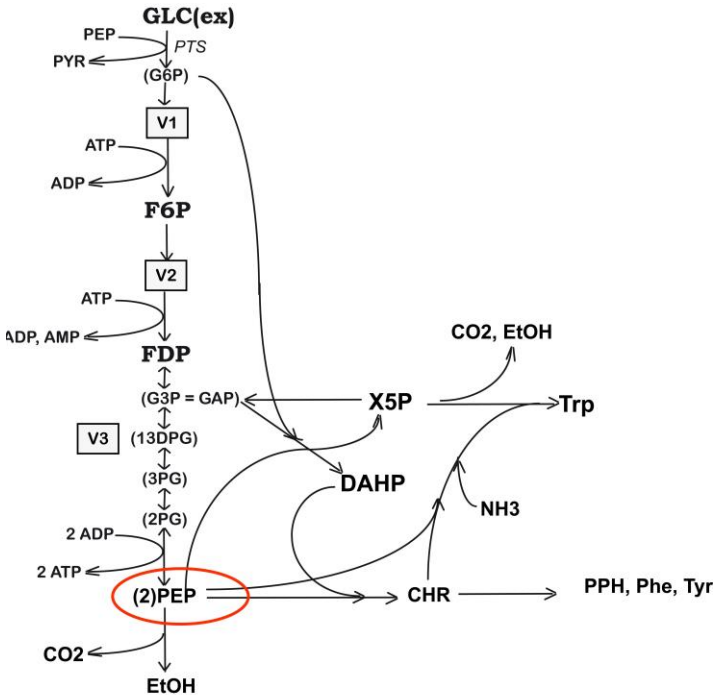

Fig. 1. Reaction pathway of TRP synthesis in *E. coli* according to [4-5], used to derive the reduced kinetic model of TRP synthesis. "Connection to glycolysis is realized through the PEP node" [4].

The TRP synthesis kinetic model of Table 1 was completed with terms accounting for linking with PEP node, PEP being included in the TRP reaction rate (see dc_T / dt term in Table 1; "the nitrogen source in the TRP balance is considered in excess and included in the k_4 constant"[4]).

PEP concentration can vary within wide limits, according to the glycolytic oscillations, controlled by the "glucose import flux, its environmental concentration, the total A(MDT)P cell energy resources, and cell phenotype characteristics (determinant for enzymes *ATPase* involved in the ATP utilization and recovery system)" [4-6,19].

Oscillations in chemical systems represent periodic transitions in time of species concentrations. "According to Franck [13], spontaneous occurrence of self-sustained oscillations in chemical systems is due the coupled actions of at least two simultaneous processes. Oscillations sourced in a so-called *oscillation node* (that is a chemical species, or a reaction), on which concomitant rapid positive (perturbing) and slow negative (recovering) regulatory loops act. Because the coupling action between the simultaneous processes is mutual, the total coupling effect actually forms closed feedback loops for each kinetic variable

involved. There exists a well-established set of essential thermodynamic and kinetics prerequisites for the occurrence of spontaneous oscillations" [13,18].

Table 1

The TRP synthesis kinetic model of Bhartiya [11]*

Species mass balance	***Parameters [5,11]***
$\frac{dc_{MRNA}}{dt} = k_2 c_{OR} C_2(T) - k_{d2} c_{MRNA} - D c_{MRNA}$ $\frac{dc_E}{dt} = k_3 c_{MRNA} - D c_E$ $\frac{dc_T}{dt} = k_4 c_{PEP} C_3(T) c_E - \frac{gT}{T + K_g} - D c_T$ $C_1(T) = \frac{K_{i,1}^{nH}}{K_{i,1}^{nH} + T^{nH}}$ $C_2(T) = \frac{K_{i,2}^{1.72}}{K_{i,2}^{1.72} + T^{1.72}}$	Initial values: c_{OR} (t=0) = 0.01, μM ; c_{MRNA} (t=0) = 0.01, μM ; c_E (t=0) = $k_3 c_{MRNA,o}/D$, μM ; c_T (t=0) = 0.01, μM k_1 = 50, 1/min.; c_{OT} = 3.32; nM ; k_{d1} = 0.5, 1/min.; k_4 = 0.059; 1/min [Maria et al.[2018a]; g = 25, μM /min.; K_g = 0.2, μM k_2 = 15, 1/min. ; k_{d2} = 15, 1/min $K_{i,1}$ = 3.53, μM ; $K_{i,2}$ = 0.04, μM $K_{i,3}$ = 810, μM ; n_H = 1.92 Cell dilution rate. D = 0.01 1/min. [11] [experimental tests]

* completed by Maria [4-5] "to be coupled with the glycolysis model. Notations: OR = the complex between O and R (aporepressor of the TRP gene); OT = total TRP operon; MRNA = tryptophan mRNA during its encoding gene dynamic transcription, and translation; E = enzyme anthranilate synthase; T= tryptophan

"In the glycolysis case, as revealed by Termonia and Ross [14-16], glycolytic oscillations occurrence is due to the antagonistic action of two processes on regulating the V2 reaction rate that converts F6P in FDP (see reaction scheme in the Fig. 1). The glycolytic oscillation occurrence and

characteristics (period, amplitude) are influenced by both external (environmental) and internal (genomic) factors" [3,4,6].

3. Dynamics of TRP synthesis for various PEP levels

Due to glycolytic oscillations [3-6], the involved species present an oscillatory dynamics. However, to simplify the present numerical analysis, average levels of PEP have been considered. While the Bhartiya [11] experiments correspond to an average [PEP]s=1 mM, other values have been tested as well, that is 0.1, 0.5, and 2 mM. The resulted species dynamics in the TRP synthesis is presented in Fig. 2:

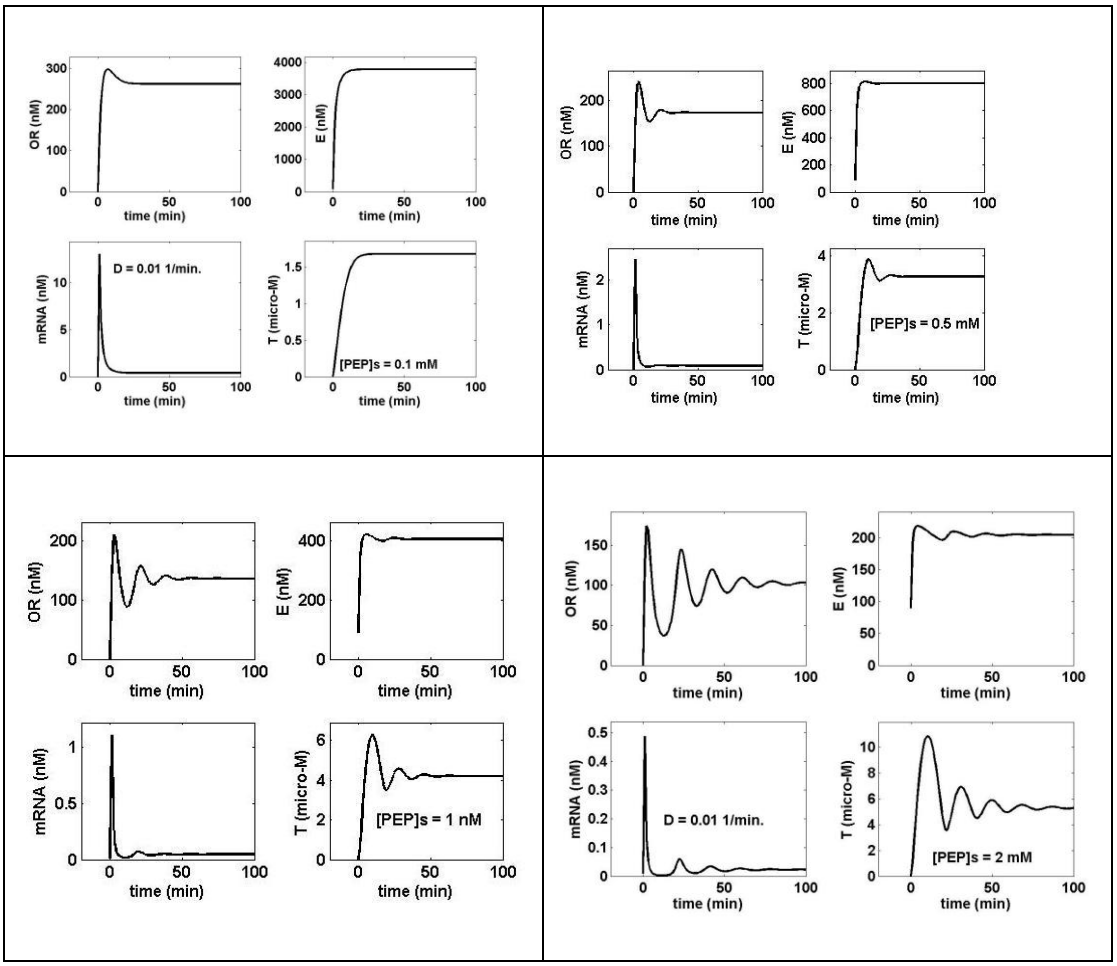

Fig. 2. Simulated dynamics of TRP synthesis (T= TRP) for *E. coli* cells with dilution of "D = 0.01 (1/min.)", that is cells with "t_C = 69.32 min.", according to the basic experiments of Bhartiya [11]

In Fig. 2, simulations have been made by adopting the followings [PEP]s stationary levels in the TRP synthesis model: [PEP]s = 0.1 mM (up-left); [PEP]s = 0.5 mM (up-right); [PEP]s = 1 mM (down-left), and [PEP]s = 2 mM (down-right).

It is to observe that PEP level has a tremendous influence on the dynamics and levels of species involved in the TRP synthesis. While for [PEP]s= 0.1 mM, the trajectories tend to stationary states of low TRP level, as [PEP]s is increasing, as the involved species present an oscillatory behavior of higher levels.

Consequently, it clearly appears that, beside cell phenotype characteristics (determinant for the TRP operon expression), glycolysis is one of the major factors determining the TRP synthesis efficiency, and TRP maximization.

As remarked by [17], glycolytic oscillations "are focused on the maintenance of energy levels in the cell (negative regulation of *PFKase* by ATP) and thus the ability to limit the conversion into energy in situations where it is not needed. Therefore, it would be more advantageous to store it or deviate the flux towards other cell cycle activities such as cell division. Consequently, mutant cells with modified enzymes activity (especially *PFKase*, *PKase*, *ATPase*, *AKase*) will lead to a noticeable modification in the metabolism, and TRP synthesis".

5. Conclusions

"The use of reduced kinetic models when modelling complex metabolic pathways is a continuous challenging subject when developing structured cell simulators for various applications (flux analysis, target metabolite synthesis optimization, *in-silico* re-programming of the cell metabolism and design of new micro-organisms, bioreactor / bioprocess optimization)"[18]. As proved by the present case study, concerning glycolysis influence on TRP synthesis in *E. coli*, reduced kinetic models, of adaptable structures to various cell types, "can successfully be used for quick analyses of cell metabolism, such as the substrate utilization, oscillation occurrence, or structured interpretation of metabolic changes in modified cells"[19].

The obtained results in the present paper prove, in a simple way that, beside cell phenotype characteristics (determinant for the TRP operon expression), glycolysis is one of the major factors determining the TRP synthesis efficiency, and TRP maximization. So, to maximize the cell TRP production, glycolysis should be speed-up.

The paper also proves in a simple, yet eloquent way, how a reduced, but structured and adequate kinetic model of an essential cell metabolic processes can support *in silico* engineering evaluations, if the cell metabolism (including metabolic by-products) will be somehow reflected by the bioreactor macro-scale dynamic model [4-6].

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