APPLICATION OF CELLDESIGNER PROGRAM FOR NUTRITION PLANNING AND FOOD SAFETY CONTROL

PRIMJENA CELLDESIGNER-A ZA PLANIRANJE PREHRANE I KONTROLU SIGURNOSTI HRANE

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Abstract

In this work the application of CellDesigner 4.0 (Systems Biology Institute, Tokyo, Japan) for nutrition planning and food safety control was tested using three models: (i) metabolic model of glycolysis, (ii) folate-mediated 1-carbon metabolism and (iii) metabolism of arsenic in human liver. Each model was simulated with a few different initial nutrient concentrations and enzyme activities. After model design and simulations in CellDesigner, it can be concluded that the use of computational tools enable fast and reproducible analysis of different input concentrations and different enzyme activity effects on specific metabolic process in the human organism. Application of computational modelling for nutrient related pathway analysis ensures a detail insight in metabolic process and simple control of the metabolic reaction affected by nutrient intake. Computational approach also simplifies prediction of potential hazards in foods, as demonstratedly metabolism of arsenic example.

Key words: CellDesigner, mathematical modelling, nutrition planning, food safety.

INTRODUCTION

Over the last few years, the implementation of systems biology in nutrition research has proven to be a very powerful tool for understanding the mechanisms by which food components influence health and/or prevent diseases. Also, systems biology simplifies the detection of biologically active molecules involved in metabolic mechanisms. Systems biology creates large data sets using and combining genomics, proteomics and metabolomics knowledge and enables a more extensive analysis of individual responses to individual nutritional interventions thus providing a comprehensive understanding of how a particular diet can affect both health and disease (Badimon et al., 2017). It is anticipated that this approach will further enlighten the molecular and biochemical interactions that take place in the cell. Acquired knowledge will lead to an improved understanding of how cellular dynamics affect tissues and the entire organism. Computer modelling provides an alternative way of solving state complexity of interactions in the cell and facilitates the representation of metabolic processes for different nutrients. There are open model-oriented databases such as the BioModels Database that contains models in SBML(Systems Biology Markup Language) format that enable the exchange in computational models (Hucka et al., 2004).

Almost all cellular processes, ranging from gene expression to synthesis and protein degradation, may be prejudiced by diet and lifestyle. Nutritive and non-nutritive components affect metabolic changes in the cell in a complex way. Similar to the mechanism of drug action, each food component affects the set of metabolic reactions acting differently depending on the components. Researches in this field are still at the beginning, and emphasis has been placed suggesting strategies to explain the effects of individual food components on the cell functions. Manipulating effects of individual food components on the cell functions could make significant improvement in the prevention and/or treatment of some chronic diseases today (Panagiotou and Nielsen, 2009).

Complementing knowledge will enable a better understanding and prediction of qualitative and quantitative correlations between a particular dietary pattern or nutrients input and their impact on health.

One of the key roles of this promising area within nutrition field is the principle of personalized nutrition according to nutritional (national) recommendations which greatly affect the treatment or prevention of metabolic disorders. Precise individual guidelines would provide a dynamic approach to classical dietary recommendations that would include parameters of the inner and outer human system that are almost constant in interactions (de Toro-Martín et al., 2017). In addition to the genetic effect, such recommendations would also include the effect of nutritional habits, nutritional behavior, physical activity, a set of microbiota and metabolic reactions.

In this work the application of CellDesigner 4.4 (Systems Biology Institute (SBI), Tokyo, Japan) for nutrition planning and...
food safety control was tested using three models: (i) metabolic model of glycolysis, (ii) folate-mediated 1-carbon metabolism and (iii) metabolism of arsenic in human liver. Each model was simulated with different initial nutrient concentrations and with different enzyme activities.

**MATERIALS AND METHODS**

**Materials**

CellDesigner version 4.4 and MS Excel 2016 (Microsoft, USA) were used in this work. CellDesigner is a computer program used to illustrate biological processes in the cell, such as graphically displaying biochemical and gene regulated networks (Klipp et al., 2010). The main features of the program include: description of biochemical semantics, detailed description of the protein state change, support of SBLM (Systems Biology Markup Language) format which describes models of biochemical reactions, an integration with SBW (SBW, Systems Biology Workbench) simulations and SBLM simulation library and possibility to browse and modify models converted from exiting databases (Funahashi et al., 2003).

**Model of the glycolysis metabolism**

Mathematical model of glucose metabolism in liver cells developed by König et al., 2012 was analysed. The model includes 36 biochemical reactions, 44 metabolites, and 185 kinetic parameters.

**Model of the folate-mediated 1-carbon metabolism**

In this paper, a mathematical model describing folate mediated 1-carbon metabolism was analysed (Reed et al., 2004). Model is based on information of folate - enzymatic kinetics and regulatory mechanisms that predict the influence of genetic and nutritional variations and describes processes in cellular cytosol. The model includes 19 biochemical reactions, 10 metabolites and 60 kinetic parameters.

**Model of arsenic metabolism in the liver**

To analyse the processes occurring in liver cells in the presence of the arsenic atom, a semi mechanistic mathematical model including the toxic-kinetic phenomena developed by Stamatelos et al. (2011) was used. The model describes the transport of arsenic via the cell membrane and arsenic metabolism in liver cells.

**Methods**

**Construction and simulation of the glycolysis metabolism model in CellDesigner**

Simulations of glycolysis metabolism were performed using the model constructed in the CellDesigner program. The effect of different initial glucose concentrations ($c_{GLC} = 4.0, 5.0, 6.0, 7.0$ and $7.7$ mmol/L) on the degradation rate of glucose in the glycolysis was investigated, as well as the effect of reduction (for $3\%$, $5\%$ and $10\%$) of the maximum reaction rates of the key enzymes included into the glycolysis pathway (glucokinase, phosphofructokinase, pyruvate kinase) on the degradation rate of glucose ($c_{GLC} = 5$ mmol/L). The results obtained by computer simulation in CellDesigner were graphically displayed using the Microsoft Excel 2016 program.

**RESULTS AND DISCUSSION**

Analysis of glycolysis metabolism model in CellDesigner

The use of CellDesigner for biochemical simulation is very widespread due to simple visualization and presentation of the logic and dynamics of complex reactions involved in most metabolic pathways (Funahashi et al., 2003). The liver glycolysis model constructed in CellDesigner is given in Figure 1. The degree of glycolysis can be determined based on measurements of the enzyme activity that catalyses the irreversible, one-way reaction: hexokinase (in the liver glucokinase), phosphofructokinase and pyruvate kinase. According to Mali et al. 2016, the activity of mentioned enzymes in diabetic patients decreases and returns to normal 24 hours after insulin therapy. Patients suffering from type II diabetes, have lower levels of insulin hormone which is a key to glucose homeostasis regulation thus leading to the conclusion that glycolysis enzymes are not completely inhibited. Their activity is reduced as much as necessary for the metabolism of smaller amounts of glucose that try to enter the cell (Lee et al., 2014). The effect of different initial glucose concentrations ($c_{GLC} = 4, 5, 6, 7$ and $7.7$ mmol/L) at the glucose degradation rate in the glycolysis was investigated. After a meal rich in carbohydrates, blood glucose concentrations increased to about $c_{GLC} = 5$ mmol/L, which is a also reference value for the period of hunger. The diagnosis of diabetes mellitus is established if glucose in the blood is over $c_{GLC} = 7$ mmol/L after overnight starving or if at any time during the day the value was greater than $c_{GLC} = 11.1$ mmol/L (Bergman Marković, 2014). Results obtained from CellDesigner simulation are presented in Figure 2, from which is apparent that the glucose concentration decreased over time.

The most significant decrease was observed for simulation with $c_{GLC} = 4$ mmol/L within the first hour. The glucose concentration decreased under $c_{GLC} = 3$ mmol/L showing the conditions when the hypoglycaemia occurs.

The glucose value of $c_{GLC} = 7$ mmol/L declined within $t = 2.5$ hours to the value of $c_{GLC} = 5$ mmol/L, which is in consistency with the literature (Bergman Marković, 2014). The results of the glycolysis simulation with the maximum glucokinase reaction rate reduced for 3%, 5% and 10% showed that reducing the maximum reaction rate slows down the glucose degradation. On the other hand, simulation results also determined that by reducing the maximum reaction rate of phosphofructokinase, well aspyruvate kinase for 3%, 5% and 10%, glucose degradation occurred faster. Phosphofructokinase is allosterically regulated by a series of effectors (ADP, AMP, cAMP, fructose-1, 6-bisphosphate, fructose-2,6-bisphosphate, ATP, citrate, phosphoenolpyruvate).
The rate of glycolysis depends on the concentration of all listed effectors. Glucose from glycogen in muscles directly enters glycolysis. The liver stores a large amount of glycogen, which is broken down as needed, and sends glucose to other parts of the body where it is missing. At a certain glucose concentration, saturating the active sites on the substrate-binding enzyme results in the substrate being no longer able to bind, thereby making the maximum reaction rate constant. The effect of reducing the maximum reaction rate (3%, 5% and 10%) of the key glycolysis enzymes (glucokinase, phosphofructokinase, pyruvate kinase) on the glucose degradation rate \( c_{0,GLC} = 5 \) mmol/L was also analysed (Figure 3).

Analysis of folate-mediated 1-carbon metabolism model in CellDesigner

Model of folate-mediated 1-carbon metabolism in the cellular cytosol constructed in the CellDesigner program is shown in Figure 4. In this study, computer program CellDesigner was used for analysis of folate metabolism which is a mediator in many other key metabolic processes such as nucleotide synthesis, DNA methylation and replication, amino acid metabolism, etc. The folate concentration is closely related to other metabolic components, whose concentration changes are dependent on the amount of folate in the cell and the metabolic activity of the responsible enzymes. Also, complexity of this metabolism is manifested in the presence of allosteric inhibition, activation and mutually interconnected reactions of two cycles (folate and methionine). Namely, many substrates within these two cycles have the role of inhibitors of their metabolic enzymes despite the fact that they do not participate directly in some chemical reactions, therefore the reaction rate and substrate concentration were not directly proportional to the cellular concentration of folate. The effect of different initial folate concentrations \( c_{0,FOLATE} = 3, 30 \) and 60 nmol/L on the concentration of formed homocysteine, tetrahydrofolate and 5-methyltetrahydrofolate was investigated. Although the concentration of serum folate is variable and dependendietary intake, a reference interval of serum folate is \( c_{0,FOLATE} = 11-57 \) nmol/L (Pagana and Pagana, 2013) thus the values of initial concentrations of folate \( c_{0,FOLATE} = 3 \) nmol/L were used for the case of extreme folate deficiency, \( c_{0,FOLATE} = 30 \) nmol/L for normal folate level and \( c_{0,FOLATE} = 60 \) nmol/L for serum folate values slightly higher than recommended. The changes of homocysteine concentration with respect to three different initial folate values are presented in Figure 5. In the first case \( c_{0,FOLATE} = 3 \) nmol/L concentration of homocysteine grows to approximately \( c_{HCY} = 0.55 \) nmol/L and after a time period of \( t = 20 \) h its concentration continues to grow. In the second \( c_{0,FOLATE} = 30 \) nmol/L and third \( c_{0,FOLATE} = 60 \) nmol/L case, the level of homocysteine reaches the highest value after an hour after which it decreases and achieves a constant value of \( c_{HCY} = 0.198 \) nmol/L and \( c_{HCY} = 0.174 \) nmol/L respectively. According to Ganguly and Alam, 2015, homocysteine is known to mediate cardiovascular problems by its adverse effects on cardiovascular endothelium and smooth muscle cells with resultant alterations in subclinical arterial structure and function. Elevated plasma homocysteine concentration is considered a risk factor for cardiovascular disease.

Given that vitamin B12 is a critical cofactor of methionine synthase enzymes, the effect of B12 deficit on the metabolism rate and the amount of its metabolites was analysed by reducing methionine synthesis activity by 10%, consistent with patients with a serious vitamin B12 deficiency.

The effect of maximum reaction rate of methionine synthesis reduction (by 10%) on concentration of produced tetrahydrofolate and is given in Figure 6. After \( t = 20 \) h, the concentration of the produced tetrahydrofolate was 84% lower than the concentration of produced tetrahydrofolate under normal conditions, whatcan be compared to a change of 73% (Reed et al., 2004).

Fig. 1. Glycolysis model constructed in CellDesigner ((1,3P2G) 1,3-bisphospho glycerate, (2PG) 2-phospho glycerate, (3PG) 3-phospho glycerate, (ACoA) acetyl-coA, (CT) citrate, (CoA) coenzyme A, (DHAP) dihydroxyacetone phosphate, (FRU16P2) fructose-1,6 bisphosphate, (FRU26P2) fructose-2,6 bisphosphate, (FRU6P) fructose-6 phosphate, (GLC) glucose, (GLC1P) glucose-1 phosphate, (GLC6P) glucose-6 phosphate, (GRAP) glyceraldehyde 3-phosphate, (LAC) lactate, (OA) oxalacetate, (MAL) malate, (P) phosphate, (PEP) phosphoenolpyruvate, (PP) pyrophosphate, (PYR) pyruvate, (UDP-GLC) UDP-glucose, (ADP) adenosine 5'-diphosphate, (AMP) adenosine 5'-monophosphate, (GTP) guanosine-5'-triphosphate, (GDP) guanosine-5'-diphosphate, (UTP) uridine-5'-triphosphate, (UDP) uridine-5'-diphosphate, (NAD) nicotinamide adenine dinucleotide)
Fig. 2. Influence of different initial glucose concentrations at the rate of glucose degradation. (---) $c_0_{\text{GLC}} = 4 \text{ mmol/L}$, (⋯) $c_0_{\text{GLC}} = 5 \text{ mmol/L}$, (→) $c_0_{\text{GLC}} = 6 \text{ mmol/L}$, (→→) $c_0_{\text{GLC}} = 7 \text{ mmol/L}$, (←-←-←) $c_0_{\text{GLC}} = 7.7 \text{ mmol/L}$

Fig. 3. Influence of changes in the maximum reaction rate of the enzymes: (a) glucokinase, (b) phosphofructokinase and (c) pyruvate kinase on the glucose degradation rate. (---) $v_{\text{max}}$ according to König et al., 2012, (⋯) $v_{\text{max}}$ reduction for 3 %, (→) $v_{\text{max}}$ reduction for 5 %, (→→) $v_{\text{max}}$ reduction for 10 %. $c_0_{\text{GLC}} = 5 \text{ mmol/L}$

Fig. 4. Model of folate-mediated 1-carbon metabolism constructed in the CellDesigner. (5,10-CH2-THF) 5,10-methylenetetrahydrofolate, (5mTHF) 5-methyltetrahydrofolate, (AICAR) aminoimidazolecarboxamide ribonucleotide, (SAH) S-adenosylhomocysteine, (SAM) S-adenosylmethionine, (THF) tetrahydrofolate, (HYC) homocysteine, (UMP) uridine-5'-monophosphate, (NADP) nicotinamide adenine dinucleotidephosphate, (Ser) serine, (Gly) glycine

Fig. 5. The effect of folate concentration change on homocysteine level (blue-initial folate concentration: $c_0_{\text{FOLATE}} = 3 \text{ nmol/L}$, orange-initial folate concentration: $c_0_{\text{FOLATE}} = 30 \text{ nmol L}^{-1}$, gray-initial folate concentration: $c_0_{\text{FOLATE}} = 60 \text{ nmol/L}$)

Fig. 6. The effect of the methionine synthesis maximum reaction rate reduction on the concentration of tetrahydrofolate (blue-reduced methionine synthesis activity by 10%, orange-normal methionine synthease activity)
Analysis of arsenic metabolism model in liver using CellDesigner

It is known from literature that arsenic represents a serious health problem and it is of great importance to increase knowledge about the impact of arsenic on the human body (Cohen et al., 2013). The use of mathematical models ensures the reduction of necessary experiments on living cells and significantly helps in planning targeted experiments (Tóth et al., 2015). According to available literature, the largest share of arsenic in the human body is ingested through drinking water. In this paper, arsenic metabolic simulations were performed in liver cells with the initial amounts of arsenic obtained on the basis of measured arsenic concentrations in the waters of Slavonia. Eastern Croatia includes the area of Slavonia, the southern part of Baranja and western Srijem. The area extends between the Drava in the north, the Sava in the south, the Danube in the east. This area is one of the areas of the Pannonian Basin, which is known for its naturally increased concentrations of arsenic in groundwater. According to some estimates, approximately 500,000 people in the Pannonian basin (Hungary, Romania, Serbia and Croatia) are exposed to arsenic concentrations higher than the maximum permissible concentration prescribed by the European Union. The increased concentration of arsenic in these areas is not a consequence of desorption processes. Arsenic in the groundwater of the Pannonian basin is mainly derived from the period of the last ice age. Changes in concentrations of extracellular and intracellular arsenic, concentrations of extracellular and intracellular monomethylated arsenic and concentrations of extracellular and intracellular dimethylated arsenite were observed over a 4000 minute time interval using CellDesigner model of the arsenic metabolism in liver, given in Figure 7. At the smallest initial amount of arsenic of $n_{As} = 0.1 \, \mu\text{moll}$, according to the literature there is no activation of the mechanism of oxidative stress in the cell (Ruiz-Ramos et al., 2009). From the simulation results, it was apparent that the fastest transfer of arsenic takes place during the first $t = 100 \, \text{min}$ of the process. It has also been observed that the amount of intracellular arsenic increases in this time interval and after that time the consumption phase occurs. A time period of $t = 100 \, \text{min}$ is probably needed to activate the enzymatic digestion/conversion of the introduced arsenic. From the simulation results for the extracellular arsenic it is apparent that after $t = 4000 \, \text{min}$ all the arsenic is located in the liver cell. The transfer of arsenic to the liver cells is carried out through the aquaporin 9 on the basis of the difference in electrochemical potential, and it is assumed that the transmission slows down as more arsenic is introduced into the cell (Liuet al., 2006).

CONCLUSIONS

After model design and conducted simulations in CellDesigner, it can be concluded that the use of computational tools allows fast and reproducible analysis of different input concentrations and different enzyme activity effect on specific metabolic process in human organisms. Application of computational modelling for nutrient based pathway ensures a detail insight in metabolic process and simple control of the metabolic reaction influenced by nutrient intake. Computational approach also simplifies prediction of potential hazards in foods, as demonstrated by the arsenic example.
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