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Mathematical Modeling of a Metabolic Network to Study the Impact of Food Contaminants on Genomic Methylation and DNA Instability

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Abstract

Environmental contamination of food is a worldwide public health problem. Folate mediated one-carbon metabolism plays an important role in epigenetic regulation of gene expression and mutagenesis. Many contaminants in food cause cancer through epigenetic mechanisms and/or DNA instability *i.e.* default methylation of uracil to thymine, subsequent to the decrease of 5-methyltetrahydrofolate (5 mTHF) pool in the one-carbon metabolism network. Evaluating consequences of an exposure to food contaminants based on systems biology approaches is a promising alternative field of investigation. This report presents a dynamic mathematical modeling for the study of the alteration in the one-carbon metabolism network by environmental factors. It provides a model for predicting "the impact of arbitrary contaminants that can induce the 5 mTHF deficiency. The model allows for a given experimental condition, the analysis of DNA methylation activity and dumping methylation in the *de novo* pathway of DNA synthesis.

Keywords

DNA-Methylation, DNA Instability, Mathematical Modeling, Logic Programming, Metabolic Network, Food Contaminant

1. Introduction

An inadequate methyl group donor enhances the risk of cancer because the one-carbon unit (CH_3) has critical functions in biological methylation reactions such as DNA-cytosine methylation, and in DNA synthesis or repair [1]-[3]. The methylation of DNA is a fundamental mechanism for epigenetic control of gene expression and the maintenance of genomic integrity, as well as uracil methylation into thymidine for the maintenance of DNA sta-

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bility to ensure chromosomal integrity during replication process [4]. Low 5-methytetrahydrofolate (5 mTHF) (**Figure 1**) reduces DNA methylation, and thymidylate synthase-mediated methylation of deoxyuridylate (dU) to thymidylate (dT). This results in a higher dU/dT ratio, increase in uracil misincorporation into DNA, inefficient DNA repair, and chromosomal breakage [5]-[7].

The role of dietary factors in DNA methylation processes diseases following folate repletion, and the consequences of genetic defect has been underlined in 2001 during the Trans-HHS Workshop in the USA [8]. Abnormal DNA methylation has been reported in disease prevention such as cancer, hepatotoxicity, pancreatic toxicity, diabetes, atherosclerosis, birth defects, and neurological disturbances. Poirier *et al.*, [9] have suggested three major causes of diseases related to methyl group insufficiency: dietary deficiency, genetic polymorphism, and a third prospective cause, chemicals. Evidence now suggest that some environmental contaminants, including exogenous chemicals such as polyhalogenated compounds, metals, some mycotoxins, and others, can produce abnormal methylation processes or abnormal folate uptake [10] linked to the etiology of diseases. Recently, Lawley *et al.* (2011) [11] demonstrates the application of a mathematical model to arsenic methylation in human studies in Bangladesh. However, in their model, various biochemical aspect of arsenic metabolism have been greatly simplified.

DNA hypomethylation and uracil misincorporation/repair are not exclusive mechanisms and both could be important in diagnostics. Since both aspects of DNA modification are strongly associated with 5 mTHF metabolism and carcinogenesis, development of methods for analyzing DNA-cytosine methylation and DNA-uracil in the aetiology of diseases is of great interest.

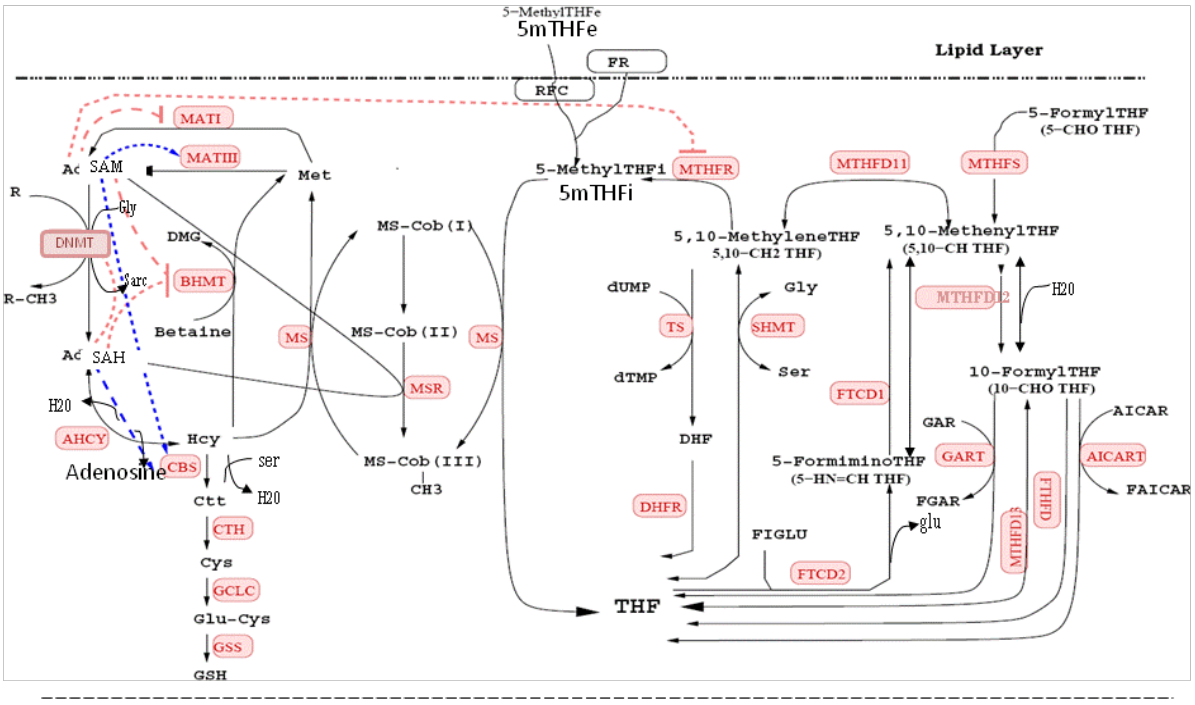

Figure 1. The Folate-mediated one carbon metabolism network [12]. Légende: Black arrow: normal reaction; Red arrow: inhibition (negative regulation); Blue arrow: activation (positive regulation). Red box: Enzyme catalyzes reaction; Black no box text: metabolites. Abbreviation**:** AHCY: S-adenosylhomocysteine hydrolase. AICART: Aminoimidazole carboxamide ribonucléotide (AICAR) formyltransférase; BHMT: betaine homocysteine methyltransferase. CBS: cystathionine beta-synthase; CTH: cystathionine gamma-lyase; DHFR: dihydrofolate reductase. FR: folate receptor; DNA MéthylTransférase; FTCD: formiminotransferase cyclodeaminase. GART: Phosporibosylglycinamide formyltransferas, phosphoribosylglycinamide synthase, phosphoribosylaminoimidazole synthase; GLCL: gammaglutamylcysteine synthase; GSS: glutathion synthase. (MATI/MATIII): adénosylmethionine transferase gene I/III. MS: methionine synthase. MSR: methionine synthase reductase; MTs: Methyltransferase MTHFD: methylenetetrahydrofolate dehydrogenase, methenyltetrahydrofolate cyclohydrolase, formyltetrahydrofolate synthase. MTHFR: 5,10-methylenetetrahydrofolate reductase. MTHFS: 5,10-methylenetetrahydrofolate synthase 5-formyltetrahydrofolate cycloligase. RFC: reduced folate; AdoMet: S-adénosylmethionine; AdoHcy: S-adénosylhomocystéine; SHMT: serine hydroxymethyltransferase; TS: tymidylate synthase. 5mTHF: 5-methyltetrahydrofolate.

2. Motivation

One of the major difficulties when working at the cellular level is describing the dynamic regulation of metabolites accurately to explain how subcellular processes such as DNA methylation or uracil methylation, are driven by environmental conditions such as 5 mTHF deficiency. Besides featuring complex networks in a living cell, with interconnecting pathways that consist of hundreds of reactions, there is need to take into account that the metabolism is subject to control and regulatory mechanisms. During the last ten years, there were many attempts to develop mathematical models for the study of cellular functions or cellular processes. We recently, built a mathematical model that integrates experimental conditions using logic programming for the study of the folate-mediated one-carbon metabolism regulation (FOCM) [12]. Here, we present the model, simulating the impact of environmental conditions such as the presence of a specific food contaminant on the network.

3. Contaminant Agents and the Alteration of Methylation Processes

Among mechanisms by which environmental agents can induce tumor formation are DNA hypomethylation associated with gene expression modification and uracil misincorporation into DNA leading to chromosomal breaks, micronucleus formation. Studies have showed that several classes of environmental chemicals that modify epigenetic marks, including metals (cadmium, arsenic, nickel, chromium, methylmercury), peroxisome proliferators (trichloroethylene, dichloroacetic acid, trichloroacetic acid), air pollutants (particulate matter, black carbon, benzene), and endocrine-disrupting/reproductive toxicants (diethylstilbestrol, bisphenol A, persistent organic pollutants, dioxin). Most studies conducted so far have been centered on DNA methylation [9] [13]. There is a need to explore the causes of many other environmental effects on methylation processes. Several paths of interaction of these contaminants on the methylation process: alteration of 5 mTHF uptake, of methyltranferase activity, or other key enzyme of the network. In this report we are interested to the interaction impinging on the availability of 5 mTHF in the cell. This is the case for instance of arsenic which may alter the expression of folate carrier and reducing the 5 mTHF uptake by cells [14].

4. Principle and Interest of Mathematical Modeling

Models describing biological systems such as the one-carbon metabolism network are too complex to be analyzed manually and therefore typically are solved numerically, using computers to solve the mathematical equations. With appropriated mathematical tools and the availability of computer-based techniques for solving equations, it is possible to predict and analyze the responses of a biological system to different conditions. In many cases the computer simulations called “dry experiments” require much lower investment and much less time compared with the typically more time-consuming and expensive biological experiments (“wet experiments”). Among benefits offer by mathematical models there are the fact that discrepancies between systems behaviors predicted by a mathematical model and actual behaviors measured in experiments can point to components that still are missing from the mathematical model, thereby assisting in developing a more comprehensive picture of a biological process. Even if it is not clear which components are missing from the system under investigation, the results obtained with the mathematical model may help to guide the design of additional experiments to clarify the issue (Systems: http://pubs.niaaa.nih.gov/publications/arh311/49-59.htm). The rationale of modeling is that it assists investigators in the analysis of the system in various possible experimental conditions. Predictions (or modeling results) are then compared to experimental measurements.

5. The One-Carbon Metabolism Network

Folate-mediated one-carbon metabolism is fundamental for cell growth and differentiation. In cells, folate uptake in the 5 mTHF form is converted to THF by transfer of the methyl group to homocysteine forming methionine and THF (**Figure 1**) [15]. Methionine can then be converted to S-adenosyl-methionine (AdoMet). AdoMet is involved in more than 100 reactions, and at least 80 AdoMet-dependent enzymes have been identified [16]. S-Adenosyl-homocysteine (AdoHcy), the by-product of methyl transfer reactions, is hydrolyzed, thus regenerating homocysteine, which then becomes available to start a new cycle of methyl-group transfer. In most tissues, homocysteine is re-methylated back to methionine through two pathways: the methionine synthase/methionine synthase reductase (MS/MSR) pathway. In the folate cycle, the THF reacts with serine synthetizing $N_{5,10}$-methyleneTHF (5,10-CH_2-THF) in a reaction catalyzed by serine hydroxymethyltransferase (SHMT). The 5,10-CH_2-THF is reduced into 5-CH_3-THF by the enzyme methylenetetrahydrofolate reductase (MTHFR), or oxidized into

5,10-methenylTHF by a reversible reaction catalyzed by methylenetetrahydrofolate dehydrogenase (MTHFD). 5,10-methenyl-THF can be converted to 10-formyl-THF by 5,10-methenyl-THF cyclohydrolase. Folate serves for DNA methylation in the transmethylation pathways [17], for synthesis of purines and a pyrimidine nucleoside (thymidine). It provides carbon units for *de novo* purine and thymidylate biosynthesis. Purine biosynthesis requires 10-formyl-THF for the C2 and C8 carbons of the purine ring catalyzed by glycinamide ribonucleotide transformylase (GART). Thymidylate biosynthesis requires CH_2-THF for the reductive methylation of deoxyuridylate catalyzed by the enzyme thymidylate synthase (TS).

The OCM system, presented in the **Figure 1**, considers an extracellular 5 mTHF uptake and a set of enzyme reactions. We considered a "generic" mammalian cell OCM including 26 enzymes reactions (*i.e.* 30 variables and 27 reactions involved). The model emphasises three functional units: one for the folate uptake and internalisation, one for the folate cycle, and one for methionine/homocysteine cycle. Each unit consists of a pool of interconnected metabolites by enzymatic reactions. The set of these reactions produces a system that we simulate using a mathematical framework. Mathematical model and reaction kinetic laws involved in the OCM model were reported in our previous study [12]. We modeled the kinetics of reactions of the OCM system using Ordinary Differentials Equations (ODE). Metabolites represent the system variables and variations of their concentration indicate the difference between their synthesis and catabolism levels.

6. Experimental Conditions and Logic Programming

We have proposed the use of the logic programming that represents an automatic way to study the possible impact of exogenous contaminant on the OCM network [12]. It consists of an automatic generation of a model based on data and knowledge available. This approach, developed in the laboratory takes into account several experimental conditions, which are considered as a set of constraints, in addition to the kinetic descriptions and as an original contribution to the standard mathematical modeling.

In the logic programming we encoded the set of data as a set of rules or facts. Each rule is encoded in the form "*IF* condition *THEN* conclusion". The first approach allows to study the structure of the biological system of interest, whereas the second proposes an analysis of the qualitative properties. As a complementary approach, we propose herein to apply such a theoretical framework for estimating the values of parameters in accuracy with the available experimental knowledge. The kinetic rates and known parameters (P') were inferred by using the logic programming inference as follow. All existing data (including experiments and literature based knowledge) are gather in a knowledge base (KB). We proceed to an inference by a back-tracking technique using the logic programming tool. In the KB, data are spread into a fact base (FB), that represents encoded biological knowledge (extracted from the literature) of our model; and the rule base (RB) that sums up the biological conditions monitoring the system behaviours. Formally, a given biological knowledge BK_i consists of sets of facts $F_i = \{F_{ij}\}$ and rules $R_i = \{R_{ij}\}$, where F_{ij} and R_{ij} are respectively atomics fact and rules obtained from BK_i. At first, the KB is empty and noted KB = { } (null). For each biological knowledge BK_i, KB is updated using a simple unification principle when a novel biological knowledge is added. The Fact Based (FB) is then completed by the new Fact set F_i and the Rules Base (RB) by the R_i ones.

The constraints are either the biological referential in which the OCM is observed (*i.e.* tissue, cell, etc...), the clinical conditions (*i.e.* healthy or pathological), and if needed, experimental parameters (*i.e.* temperature and pH of *in vitro* enzyme reactions). Each constraint represents a condition in which the model must behave accurately with given known experimental conditions. Logic programming is a well-investigated domain of machines learning artificial intelligence. Logical constraints are used to verify biological model behaviors during environmental perturbations via model-checking methods. The set of data is encoded as a set of rules or facts which gives rise of the opportunity to automatically learn novel rules by induction or deduction. Each rule is encoded in the form "*IF* condition *THEN* conclusion". The first approach allows studying the structure of the biological system of interest, whereas the second proposes an analysis of the qualitative properties. As a complementary approach, we propose herein to apply such a theoretical framework for estimating the values of parameters accurately with the available experimental knowledge. The parameters identification, inference of known parameters and the model validation are reported elsewhere [12].

7. Simulating the Impact of Contaminants by Reducing 5 mTHF Level in the Network

Food contaminants such as arsenic, fumonisin B1 and other, decreases intracellular 5 mTHF pool by reducing

the activity of 5 mTHF transporter [10] [14]. In a previous in vitro study using HepG2 cell line grown either in experimental complete medium or in folate-depleted medium, we shown global hypomethylation of genomic DNA induced by the absence of folic acid in folate-depleted medium and significant increase of uracil residues in the same DNA sample [15].

We simulate the impact of arbitrary contaminants that can induce the 5 mTHF deficiency. 5 mTHF was computed by testing 50% and 25% of extra cellular 5 mTHF (5 mTHFe) input compared to 100% (10 nM[1]) (**Figure 2**). With 25% of 5 mTHFe major metabolites were quite insensitive while other metabolites show differential behavior. About AdoHcy, the methyl group donor, we observe an inverse tendency. For 50% and 100%, there was decreasing from 22.87 Units to 5.21 Units at 0.27 h and rapid increase to 25.12 Units at 1h of running, then a second level of rapid increasing to 88.97 Units at 1.5h was observed. With 25%, AdoHcy shows a different behavior from 2.5 Units increasing slowly to reach 18.94 Units at 2.6 h to remain constant. Finally, the curve of AdoMet/AdoHcy ratio increased at the beginning of running, reaching the highest level at 0.42 h with a ratio of

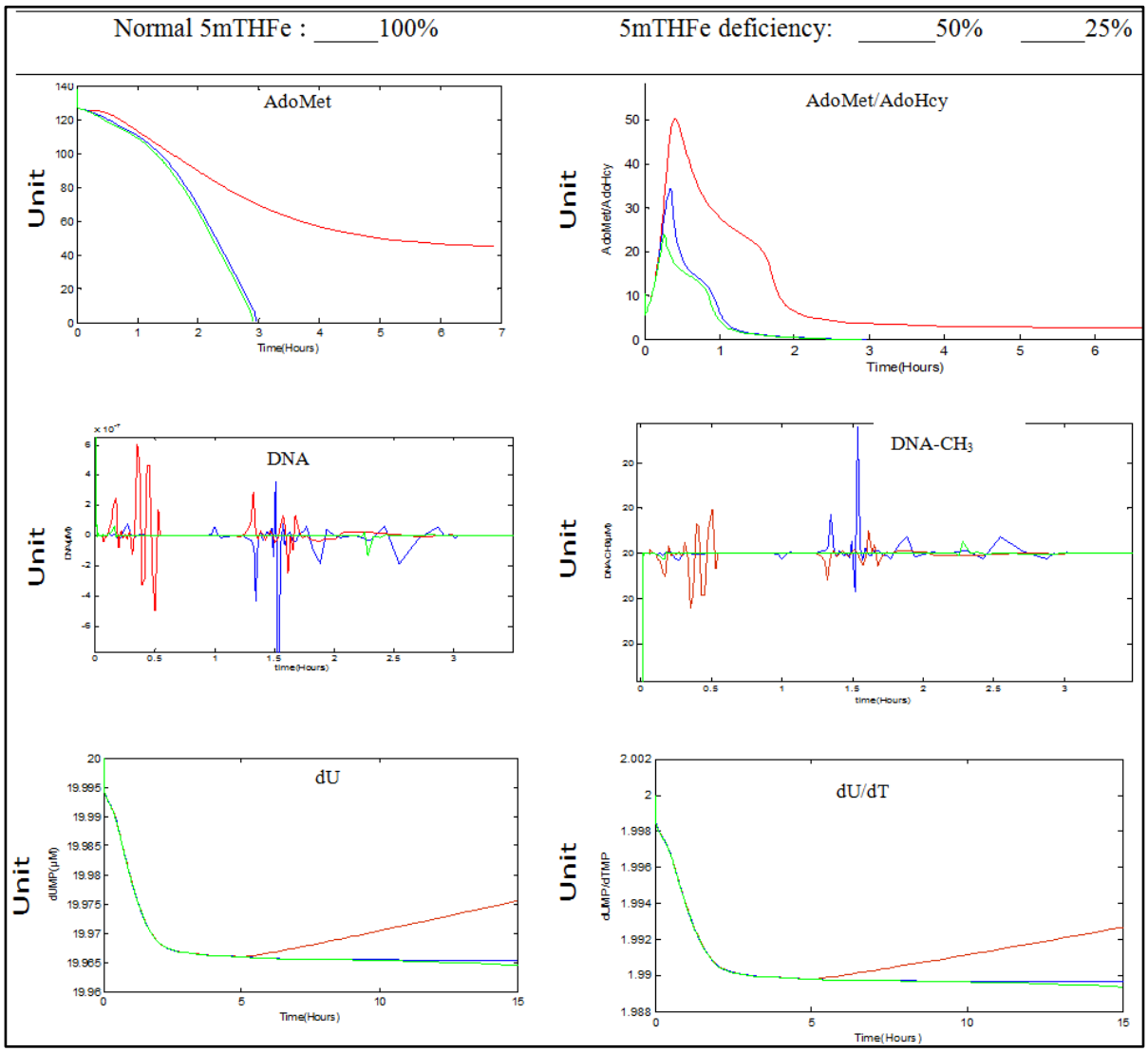

Figure 2. Result of simulating 5-methyltetrahydrofolate (5mTHF) deficiency [12]. Figure shows the variations relate to the transmethylation pathway, and the uracil methylation pathway. Disruptions of these subsystems are represented here by the behavior of state variables (metabolite concentrations over time). Thus, we observe an overall weak disturbance between the state without deficit (100% green) and the state with 50% deficit (blue). But the disruption is greater with 25% (red). Abbreviation AdoMet: S-adenosylmethionine; AdoHcy: S-adénosylhomocystéine: dU: deoxyuridylate, dT: thymidylate.

[1]This value represents also the simulation units (Units) that was at the nanomolar scale for 5mTH and other folate derived metabolites.

22; 34 and 50 respectively for 100%; 50% and 25% of 5 mTHFe condition respectively. In regard of the trans methylation reaction, results of time course DNA methylation process did not show characteristic differential behaviours with the three 5 mTHFe conditions. However, at steady state there are two oscillations of variable amplitude. These oscillations show lower amplitudes on methylated DNA (DNA-CH_3). The observed oscillations are not clear. However it is interesting to note that physiologically folate deficiency produce both global genomic DNA hypomethylation and gene specific hyper methylation. For dU, after a rapid decreasing with the three 5 mTHFe conditions, we observed a progressive increasing with 25% condition from 19.96 Units at 5h of running to 19.97 Units at 15 h of running. Finally with 100% of 5 mTHFe, dU/dT ratio reaches the steady-state 1.95 after 2.5 h compared to 1.99 after 2 h with 25% of 5 mTHF.

Finally, the model demonstrates that dependent of the impact of a contaminant on the decrease of 5 mTHF pool, there are different profiles of the dynamical behavior of the network. The questions emerging from such a study is how the stability of the critical reaction in the cycle is maintained when large localized changes occur either within the system or the input, and what is the significant of observed oscillations for DNA and DNA-CH_3 curves for cell. Otherwise a recent study from Nijhout *et al.* [18] have shown the inhibition of *GNMT* by 5 mTHF and consequently the existing long-range interactions stabilize DNA methylation. This report didn't include this particular finding that will need to include in the future.

8. Conclusion

The model allowed successfully the simulation of many key regulatory processes in one-carbon metabolism network. However it may be refined and used as tool in predictive nutritional toxicology to provide novel hypotheses for pathogenesis. It can be a predictive tool and could, therefore, be substituted in the future to experimental techniques in some cases.

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