A Model of Phospholipid Biosynthesis in Tumor in Response to an Anticancer Agent in Vivo

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Summary

We study, in this paper, a model for the core of the system of the Glycerophospholipid metabolism in the murine cells. It comprises the simple and enzymatic reactions of PhosphatidylEthanolamine and the PhosphatidylCholine . The model's general structure is taken from a number of books and articles. We translate this model into a set of ordinary differential equations (ODEs), to propose a quantitative explanation of the experimental experiences and the observed results. In order to make it usable as a basis for simulations and mathematical analysis we need to make precise the various constants present in the equations but which are usually not directly accessible in the literature. In a first step we considered experimental data of rat's liver cells obtained by NMR spectroscopy: given the values of metabolite concentrations we find appropriate parameter values which allow us to describe the system with ODEs. We have then performed several analyses using the developed model such as stability analysis. A first interesting result is the global stability of the system which was observed by simulation and then proved by mathematical arguments. A second important result is that we observe on the diagrams that the steady state for normal cells is precisely a singular point of order two, whereas tumoral cells present different characteristics; this fact has been proved for PhosphatidylEthanolamine N-Methyl transferase (PEMT), an enzyme which seems to be identified for the first time as a crucial element in the tumoral process. In a second step we applied our model to experimental data of proton HRMAS NMR spectroscopy for solid B16 melanoma and Lewis lung (3LL) 3LL carcinoma cells treated by Chloroethyl Nitrosourea (CENU). We performed a complete comparative analysis of parameters in order to learn the predictive statements to explain increases and decreases which one can observe in concentrations.

1 Introduction

Phospholipids are a major component of biological membranes. They are a class of lipids formed from four components: fatty acids, a negatively-charged phosphate group, alcoholamine and a backbone. PhosphatidylCholine (PtdCho) and PhosphatidylEthanolamine (PtdEth) are two of the most abundant Phospholipids. In most eukaryotic cells, PtdCho is synthesized through two different pathways [1]; in the cytidine diphosphate-choline (CDP-choline)

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pathway (Kennedy pathway) and via the transmethylation of PtdEth catalysed by Phosphatidyl-Ethanolamine N-Methyl transferase (PEMT). Choline, supplied by food, is principally in the form of PtdCho but also exists as free Choline [2, 3].
The Kennedy pathway for producing PtdCho, involves the activation of Choline (Cho) to CDP-choline through an intermediate product, PhosphoCholine (P-Cho). The second pathway to produce PtdCho consists of three sequential methylations of PtdEth. Cho derived from turnover of PtdCho produced by the methylation pathway is used for PtdCho synthesis through the Kennedy pathway. Therefore the activity of the Kennedy pathway does not reduce even in the absence of Cho in the growth medium [4].
In 1975, Sundler *et al.* used radioisotope methods to examine the rates of synthesis for PtdCho and PtdEt of liver [5, 6]. In their study, there are still questions about metabolic pathways to be answered. However evidence of the two different pathways of PtdCho synthesis and the relative activities of these pathways was provided by Vance et al. [7, 8]. The Nuclear Magnetic Resonance (NMR) spectroscopy method has been used to study the biosynthesis of PtdCho and PtdEth [9, 10]. The NMR technique can also provide a detailed examination of the specific metabolic pathways. Reo *et al*. performed kinetic analyses of liver PtdCho and PtdEth biosynthesis using ^{13}C NMR spectroscopy [11].
The development of methods for pathway-specific analyses of phospholipid biosynthesis in intact tissue can help in our understanding of numerous cellular processes, and may be important for cancer studies. This is why the Phospholipid metabolism has attracted the attention in cancer research. It is of interest to biologists to be able to follow the phospholipid metabolism in circumstances in which cell survival and cell proliferation are of concern, *e.g.* neurological disorders and cancer [12, 13]. Thus there is a need to develop a model for their biosynthesis and turnover. This is why we tried to find a model for the GlyceroPhospholilid metabolism in the murine liver cell. Our goal is to build a model with which one could simulate the behavior of Phospholipids interactions. Due to the complexity of this system, mathematical modeling and numerical simulation is necessary to enable a compact representation of the current knowledge and to make meaningful quantitative predictions guiding future experimental studies.
Once the model is developed to represent the metabolism of the murine liver cell, one can apply it to study the Phospholipid metabolism of mouse melanoma and 3LL carcinoma cells. In the recent years several studies have been carried out to perform chloroethylnitrosourea (CENU) chemotherapy for the treatment of B16 melanoma and Lewis lung (3LL) carcinoma tumors in vivo [14, 15, 16, 17, 18]. We apply our model to study the effects of such treatments. For each of these two tumors we have experimental data for three different phases: Control(CTL), Inhibition(INH) and Recovery(REC)[27].The results of our comparative analysis, based on our simulation, show good agreements with experimental data [16].
In our study, we have directly translated the biochemical reactions into ODEs, following the Michaelis-Menten chemical paradigm. Other authors have used probabilistic models or computer science models based on π-calcul; under very reasonable assumption all these models yield ODEs as stated by L. Cardelli in his series on Artificial Biochemistry [19].

2 Methods

In this section, we first describe our model for the Phospholipids' metabolism which is supplied from bibliographical references(e.g. M.Israel and L.Schwartz [20, 30, 31, 32, 33, 34]). Then we introduce our methods to obtain experimental data. Next, we develop an ODEs-based model. Finally, we study different phase spaces of the system.

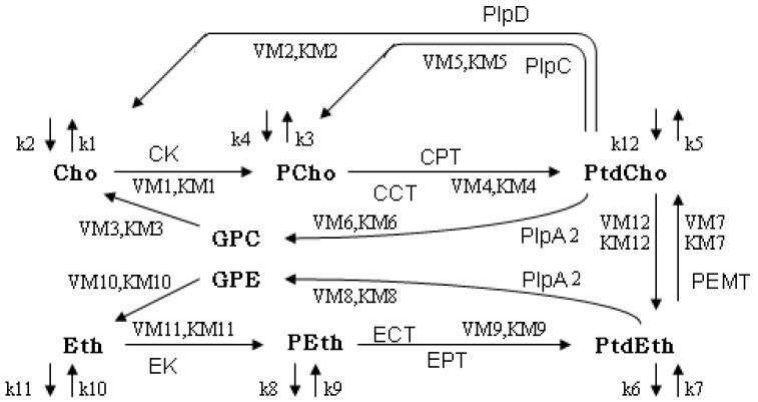

Figure 1: Schematic representation of the model. Arrows with VM_i and KM_i parameters refer to enzymatic reactions while the rest represent simple reactions. Reactants: Cho(Choline), PCho(Phospho-Choline), PtdCho(Phosphatidyle-Choline), GPC(Glycero-PhosphoCholine), Eth(Ethanolamine), PEth(Phospho-Ethanolamine), PtdEth(Phosphatidyle-Ethanolamine), GPE(Glycero-PhosphoEthanolamine). Enzymes: CK(Choline-Kinase), EK(Ethanolamine-Kinase),CPT(PC-transferase),CCT(PhosphoCholine-Cytidyl-Transferase), ECT/EPT(PhosphoEthanolamine-Cytidyl-Transferase), PEMT(PhosphatidyleEthanolamine-N-methyl-Transferase),PlpA2 (PhosphoLipase A2), PlpC(PhosphoLipase C), PlpD(PhosphoLipase D). Parameters: VM(Michaelis maximum reaction rate), KM(Michaelis concentration constant), k_1-k_{12}(Rate constants for external reactions).

2.1 Biochemistry of the phospholipid metabolism

Our analysis concerns twenty-four biochemical reactions (Fig. 1). In this system there are two main sub-systems with similar reaction structures; the first one is the Choline (Cho) cycle and the second one is the Ethanolamine (Eth) cycle. In order to have a more complete model several reactions involving external reactants are also considered in the model (Fig. 1).

Choline cycle: Cho is phosphorylated in a reaction catalyzed by Choline-Kinase (CK), resulting in the formation of PhosphoCholine (PCho)[30]. PCho is converted to PtdCho in a two step reaction, first catalyzed by PhosphoCholine-Cytidyl-transferase (CCT), then by PC-transferase (CPT)[30, 31]. PtdCho is converted to Glycero-PhosphoCholine (GPC) in the reaction catalyzed by Phospholipase A2 (PlpA2)[31]. In addition, PCho and Cho can be synthesized from hydrolysis of PtdCho through the reactions catalyzed by Phospholipase C (PlpC) and Phospholipase D(PlpD) respectively[31, 32]. Cho can be also synthesized from GPC[32].

Ethanolamine cycle: Eth is phosphorylated in an enzymatic reaction catalyzed by Ethanolamine-Kinase (EK), resulting in the formation of PhosphoEthanolamine (PEth)[33]. PEth is converted to PtdEth in a two steps reaction, first catalyzed by PhosphoEthanolamine-Cytidyl-transferase (ECT), then by PE-transferase (EPT)[33, 34]. PtdEth is converted to Glycero-PhosphoEthanolamine (GPE) in the reaction catalyzed by Phospholipase A2 (PlpA2)[8, 31]. Eth is synthesized from GPE[32].

The above two sub-systems are related through the reaction between PtdEth and PtdCho where PhosphatidylEthanolamine N-Methyl Transferase (PEMT) plays the role of catalyst[4, 7, 8]. This reaction seems to be an important reaction in this system, and the basis of main analysis in our study, since homeostasis of PtdCho is essential to maintain cell survival.

External reactions: In addition to the reactions described so far, most of the reactants in phospholipids metabolism model have external reactions. For example there is a reversible reaction in which PhosphatidylSerine (PtdSer) releases CO_2 and PtdEth as products [21]. In the same way there are several external reactions in which Cho, Eth, PCho, PEth, PtdCho and PtdEth have the role of substrate or product. We present these external reactions by input or output arrows in the model(Fig. 1)[9, 21, 30].

2.2 Mathematical simulation

The mathematical simulation of the model is performed via a free mathematical software, Scilab. For the simulation of the biological system we use traditional reaction-rate approach by defining the equations describing the system and setting the initial parameters required for the calculation. In this approach, the chemical reactions are modelled by ordinary differential equations (ODEs) representing the concentrations of the substances. We formulate the basic model for the given chemical reactions in terms of a system of differential equations , which consists of one differential equation for the kinetics of each of the reactants. In each equation, [X] represents the concentration of a given reactant X, whose values are expressed in $\mu mol.g^{-1}$. The k_i is the reaction rate coefficient or rate constant in simple reactions. For enzymatic reactions the parameters VM_i and KM_i are the maximum rate and Michaelis constant respectively. The model writes as follows:

$$\frac{\partial}{\partial t}[Cho] = k_2.e^{-[Cho]} + \frac{VM_2.[PtdCho]}{KM_2 + [PtdCho]} + \frac{VM_3.[GPC]}{KM_3 + [GPC]} - \frac{VM_1.[Cho]}{KM_1 + [Cho]} - k_1.[Cho] \quad (1)$$

$$\frac{\partial}{\partial t}[Eth] = k_{10}.e^{-[Eth]} + \frac{VM_{10}.[GPE]}{KM_{10} + [GPE]} - \frac{VM_{11}.[Eth]}{KM_{11} + [Eth]} - k_{11}.[Eth] \quad (2)$$

$$\frac{\partial}{\partial t}[PCho] = k_4.e^{-[PCho]} + \frac{VM_1.[Cho]}{KM_1 + [Cho]} + \frac{VM_5.[PtdCho]}{KM_5 + [PtdCho]} - \frac{VM_4.[PCho]}{KM_4 + [PCho]} - k_3.[PCho] \quad (3)$$

$$\frac{\partial}{\partial t}[PEth] = k_9.e^{-[PEth]} + \frac{VM_{11}.[Eth]}{KM_{11} + [Eth]} - \frac{VM_9.[PEth]}{KM_9 + [PEth]} - k_8.[PEth] \quad (4)$$

$$\frac{\partial}{\partial t}[PtdCho] = k_{12}.e^{-[PtdCho]} + \frac{VM_4.[PCho]}{KM_4 + [PCho]} + \frac{VM_7.[PtdEth]}{KM_7 + [PtdEth]} - \frac{VM_6.[PtdCho]}{KM_6 + [PtdCho]} - \frac{VM_5.[PtdCho]}{KM_5 + [PtdCho]} - \frac{VM_2.[PtdCho]}{KM_2 + [PtdCho]} - \frac{VM_{12}.[PtdCho]}{KM_{12} + [PtdCho]} - k_5.[PtdCho] \quad (5)$$

$$\frac{\partial}{\partial t}[PtdEth] = k_7.e^{-[PtdCho]} + \frac{VM_{12}.[PtdCho]}{KM_{12} + [PtdCho]} + \frac{VM_9.[PEth]}{KM_9 + [PEth]} - \frac{VM_8.[PtdEth]}{KM_8 + [PtdEth]} - \frac{VM_7.[PtdEth]}{KM_7 + [PtdEth]} - k_6.[PtdEth] \quad (6)$$

$$\frac{\partial}{\partial t}[GPC] = \frac{VM_6.[PtdCho]}{KM_6 + [PtdCho]} - \frac{VM_3.[GPC]}{KM_3 + [GPC]} \quad (7)$$

$$\frac{\partial}{\partial t}[GPE] = \frac{VM_8.[PtdEth]}{KM_8 + [PtdEth]} - \frac{VM_{10}.[GPE]}{KM_{10} + [GPE]} \quad (8)$$

In this model, each of these differential equations expresses the rate of change of one reactant as a sum of fractional terms for enzymatic reactions and non-fractional terms for simple reactions. Furthermore, we proposed an exponential formula with concentrations of studied reactants in our system as variables, to explain the kinetics of reactions with reactants from the external environment. Diffusion phenomena are the reason of this exponential form. Molecular

diffusion, often called simply diffusion, is a net transport of molecules from a region of higher concentration to one of lower concentration by random molecular motion.

2.3 Model analysis for healthy liver cells

In this section, we use the described mathematical model in two steps: first we try to find the different required parameters in system of equations, such as the rate constants for each reaction, using the experimental values of the concentrations in healthy rat liver metabolism. The second step is to study the phase spaces diagrams and also the different stability analyses using the parameters obtained for healthy liver cells.

2.3.1 Concentrations and Parameter estimations

The described kinetic equations require different parameters, such as the rate constants for each reaction. In the first application of our mathematical model, the experimental values that we use, are derived from the concentrations of rat liver metabolism measured at several instants during infusion with Choline and Ethanolamine [11]. Concentration of [PtdCho] and [PtdEth] were measured from the ^{31}P NMR spectra of the lipid extracts [11]. A description of these experimental analysis is given in [23, 24]. The parameter values have an important effect on the precision of the model which is representing this biological system. However these values, k_i, VM_i and KM_i, are difficult to estimate experimentally and many are unknown; that is why we estimate them by means of a numerical method. There are several ways of doing so. Since the changes in the concentration values in the different instants are small, we predict that the system is initially close to its steady state. We use this assumption to treat our system of ODEs as a nonlinear algebric problem (since all derivatives are zero).This simplification now leaves a system of equations with a number of variables greater than the number of equations. Therefore mathematically we have a set of possible solutions of this ODE system. Furthermore we know that the vector of these rate constants needs to insure the behavior of the model in such a way that the cell is viable. For example there exists a specific limited range of concentration, for some metabolites, in which the cell can stay alive. These ranges can give upper and lower bounds for parameters.[25]. Once we take into account all these biological constraints, the possible rate constant vectors fall into a subset of the parameter space. Characterizing this subset would be a prediction of the model, and so would be characterizing the set of all the dynamics of the model consistent with the parameter vectors in this subset. The vector of parameters which are shown in Table 2 (*cf.* Annexe 1) is one of these possible solutions. To obtain this, we first define the sum of squares of rate equations of section 2.2 as a function. Then we find a solution which minimizes the value of this function, in the viable range for parameters. For this aim, since we have two inequality constraints as a lower bound and an upper bound for each of parameters, we use the Karush-Kuhn-Tucker(KKT) theorem [35]. The KKT conditions are necessary for a solution in nonlinear programming to be optimal, provided some regularity conditions are satisfied. It is a generalization of the method of Lagrange multipliers to inequality constraints[35]. This method gives a solution that best fits with the biological measurements. Table 1 (*cf.* Annexe 1) represents the average of concentrations which are measured experimentally, for 8 reactants of our system. We first try to use the average values presented in Table 1 (*cf.* Annexe 1), to obtain a possible vector of the constants of reactions k_i, the maximum velocity and kinetic constant of Michaelis-Menten model VM_i and KM_i (Table 2 (*cf.* Annexe 1)). We also try to find a possible vector of the constants, for each set of concentrations measured in each of 6 time points [11]. Comparing the vectors obtained in each of these two cases, we do