ANALYSIS OF THE STEADY-STATE RELATIONS IN MATHEMATICAL MODEL OF CHOLESTEROL BIOSYNTHESIS

Aleš Belič[1], Jure Ačimovič[2], Marko Goličnik[2]

[1] University of Ljubljana, Faculty of Electrical Engineering
1000 Ljubljana, Tržaška 25, Slovenia
[2] University of Ljubljana, Faculty of Medicine, Institute of Biochemistry
1000 Ljubljana, Vrazov trg 2, Slovenia

ales.belic@fe.uni-lj.si(Aleš Belič)

Abstract

In European Union 10-15% of population has elevated levels of cholesterol, which is know to be a risk factor for cardiovascular diseases. Several treatment strategies and drugs have been developed to control the elevated cholesterol levels, however, they are not always successful. Statins are now most widely used cholesterol-lowering drugs, however, all the mechanisms of their action are not understood and can sometimes lead to adverse effects. A dynamical mathematical model of the cholesterol biosynthesis network was developed to study the effects of various substances that interfere with cholesterol biosynthesis. In this article we show that in spite of serious lack of data, the model can be used to study the concepts of possible mechanisms of cholesterol biosynthesis and drug interactions. If only steady-state data is used for model identification the model can predict steady-state relations in different situations, while dynamical properties cannot be correctly simulated. However, the model can be improved if dynamical data becomes available. The performed experiments that were analysed with the model simulations show, that the two substances with completely different modes of action most likely trigger the same control mechanism.

Keywords: Modelling, Simulation, Cholesterol, Systems Biology, Functional Genomics

Presenting Author's Biography

Aleš Belič received B.Sc and Ph.D. degrees in electrical engineering from the University of Ljubljana, Slovenia in 1994, and 2000 respectively. He is currently Associate Professor at the Faculty of Electrical Engineering, University of Ljubljana. Main areas of his professional interest are artificial intelligence modelling techniques in bio-medical areas. Currently he is involved in modelling of cholesterol pathways in human in the frame of 6th European Framework project STEROLTALK, and in functional analysis of EEG signals.

1 Introduction

In European Union 10-15% of population has elevated levels of cholesterol, which is know to be a risk factor for cardiovascular diseases [1]. While the relation between cholesterol and cardiovascular diseases is not clear, it can be statistically shown, that elevated levels of blood cholesterol are related to cardiovascular diseases. Cholesterol and glucose metabolisms are also closely related, therefore, imbalance in cholesterol metabolism can also disturb the homeostasis of glucose metabolism, which may complicate the underlying disease [2]. Consequently, any interaction with any metabolic pathway may cause system-wide disturbance and unpredictable effects. That is why safe-drug development is so problematic [3]. Identification of metabolic networks is extremely difficult, since they all operate in closed loops and since they constantly interact with each other. Several methods have been developed in order to solve the problem [4, 5, 6, 7, 8, 9, 10, 11, 12, 13], however, the complexity of the problem prevents clear identification of the system.

Sterol metabolism is one of the key metabolic processes in the organisms and cholesterol is the main building block for the cell membranes of animals; however, the biosynthesis metabolic network is still relatively poorly understood. While any animal cell is capable of producing cholesterol, liver is the major cholesterol-metabolising organ in the body. Several drugs have been designed to interact with cholesterol biosynthesis with goal to lower blood cholesterol levels. Statins are now among widely used cholesterol lowering drugs, however, in some cases, severe adverse effects, such as rhabdomiolysis, have been reported. The cholesterol metabolic network begins with acetyl co-enzyme A which is one of the basic building blocks in many metabolic processes, including Krebs cycle. After several enzyme reactions squalene is formed and from squalene lanosterol, the first sterol (cyclic molecule) is formed. From lanosterol to cholesterol the networks becomes extremely complicated (see Fig. 1). Before squalene several other pathways separate from the cholesterol network, leading to other important metabolites, such as isoprenoids, co-enzyme Q etc.. Some intermediates between lanosterol and cholesterol are also substrates for other pathways, such as 7-dehydro cholesterol which is a substrate for vitamin D biosynthesis. Cholesterol and also some metabolites of the late cholesterol metabolic network can activate SREBPs which regulates the expressions of all enzymes involved in cholesterol biosynthesis [14, 15]. The described structure of the network is typical biological feedback mechanism. Statins have been designed to inhibit HMGCR enzyme which is of the early enzymes in the cholesterol network, however, they may interact with other processes as well. In understanding the functioning of such a complex system, modelling and simulation can contribute a valuable information. Therefore, an attempt was made to model the late part of the cholesterol network in order to evaluate the effects of statins and some other xenobiotic substances. However, due to lack of suitable data (statical relations before and after drug administration) the model parameters could not be identified. In spite of ad-hoc selected parameter values, the model is still a valuable tool for cholesterol network functioning research. The objective of the paper is to show what aspects of the unidentified model can serve as information on real system functioning in steady-state.

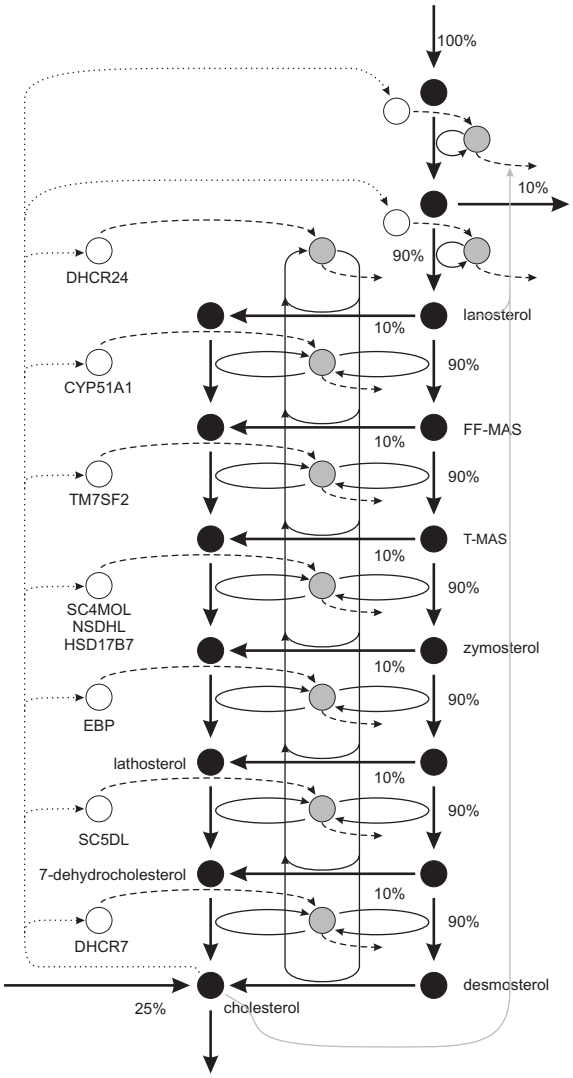

Fig. 1 Structure of the cholesterol synthesis mathematical model. The meaning of the symbols is as follows: black circles metabolites, grey circles enzymes, white circles mRNA, thick black lines metabolic flux under normal conditions (arrows show the normal direction of reactions), thin black lines enzymes involvement, grey lines the effect of metabolites on enzyme degradation, broken lines enzyme formation and degradation, dotted lines the effect of metabolites on gene expression, % - division of metabolic flux at branching points.

2 Dynamical model of cholesterol biosynthesis

The model was designed as dynamic model with relations between molecules as described in Fig. 1. The basic building block for modelling metabolic networks are enzyme reactions (see Fig. 2 and thick arrows in Fig. 1). Each enzyme reaction is described with four

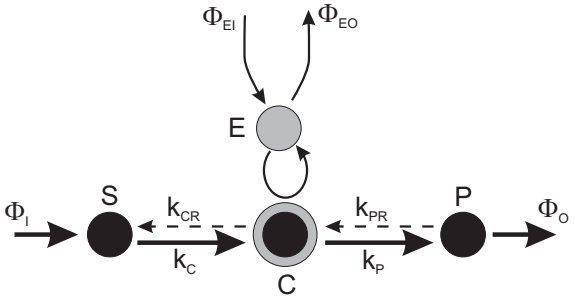

Fig. 2 Structure of enzyme reaction mathematical model. The meaning of the symbols is as follows: black circle substrate (S) and product (P), grey circle enzyme (E), grey/black circle complex (C), thick black arrows forward reaction, broken arrows - reverse reaction, thin black arrows - enzyme production and degradation, k_C, k_P - forward reaction rate constants, k_{CR}, k_{PR} - reverse reaction rate constants, Φ_I, Φ_O - metabolic fluxes in and out of the reaction, Φ_{EI}, Φ_{EO} - fluxes of enzyme production and degradation.

differential equations:

$$\frac{dS}{dt} = \Phi_I - k_C \cdot E \cdot S + k_{CR} \cdot C \tag{1}$$

$$\frac{dC}{dt} = k_C \cdot E \cdot S + k_{PR} \cdot E \cdot P - k_P \cdot C - k_{CR} \cdot C$$

$$\frac{dP}{dt} = k_P \cdot C - \Phi_O - k_{PR} \cdot E \cdot P$$

$$\frac{dE}{dt} = \Phi_{EI} + k_P \cdot C + k_{CR} \cdot C - k_c \cdot E \cdot S - $$
$$- k_{PR} \cdot E \cdot P - \Phi_{EO}$$

where S denotes the concentration of the substrate, C the concentration of the complex, P the concentration of the product, E the concentration of the enzyme, Φ_I the flux of the substrate into the reactor, Φ_O the flux of the product out of the reactor, Φ_{EI} the flux of the enzyme into the reactor, Φ_{EO} the flux of the enzyme out of the reactor, k_C the rate constant of the complex formation, k_{CR} the rate constant of decomposition of the complex into the substrate and the enzyme, k_P the rate constant of the product formation, and k_{PR} the rate constant of the complex formation from the enzyme and the product. For batch reactors, where finite quantities of the substrate and the enzyme are mixed, each enzyme reaction would contribute four independent rate constants to the pool of model parameters. However, metabolic networks normally operate in a mode with continuous supply of substrates and enzymes which introduces some limitations on the choice of the model parameters values. Operation in continuous metabolic flux mode requires that the concentrations of the involved substances settle at a non-zero steady-state.

3 Analysis of the model in steady-state conditions

The measurements that were used in the study provided only information on the steady-state relations, so the model was developed and analysed for the steady-state conditions. Steady-state of the system is reached when all the time derivatives in the above equations are equal to zero and when $\Phi_I = \Phi_O$, and $\Phi_{EI} = \Phi_{EO}$. The steady-state equations of the enzyme reaction form the following system of equations:

$$0 = \Phi_I - k_C \cdot E \cdot S + k_{CR} \cdot C \tag{2}$$
$$0 = k_C \cdot E \cdot S + k_{PR} \cdot E \cdot P - k_P \cdot C - k_{CR} \cdot C$$
$$0 = k_P \cdot C - \Phi_O - k_{PR} \cdot E \cdot P$$
$$0 = \Phi_{EI} + k_P \cdot C + k_{CR} \cdot C - k_c \cdot E \cdot S - $$
$$- k_{PR} \cdot E \cdot P - \Phi_{EO}$$

The concentrations of S, E, C, and P can be independently normalised to the dimensionless quantities by dividing their values by their steady-state values. At the same time also the reaction rates are scaled by the steady-state values of S_N, E_N, C_N, and P_N. The normalised reaction rates now represent the steady-state contributions of the specific reaction steps on the whole metabolic flux of the enzyme reaction. Using the steady-state normalised values of S_N, E_N, C_N, P_N, and the normalised reaction rates the equations above can be rewritten. Let us here present only the equation describing the steady-state of C_N since it represents the relation between all the free model parameters:

$$0 = k_{CN} \cdot E_N \cdot S_N + k_{PRN} \cdot E_N \cdot P_N - \tag{3}$$
$$- k_{PN} \cdot C_N - k_{CRN} \cdot C_N$$

To make the relation between the forward and the reverse metabolic fluxes more transparent, the following ratios can be defined:

$$\frac{k_{PRN}}{k_{CN}} = r_1 \tag{4}$$
$$\frac{k_{CRN}}{k_{PN}} = r_2$$

Thus the equations can be rewritten in a new form,

$$0 = k_{CN} \cdot E_N \cdot S_N + k_{CN} \cdot r_1 \cdot E_N \cdot P_N - \tag{5}$$
$$- k_{PN} \cdot C_N - k_{PN} \cdot r_2 \cdot C_N$$

which yields the final form

$$0 = k_{CN} \cdot E_N (S_N + r_1 \cdot P_N) - k_{PN} \cdot k_{CN} (1 - r_2) \tag{6}$$

However, E_N, S_N, C_N, and P_N are in the steady-state of the undisturbed system equal 1, which further simplifies the relation:

$$0 = k_{CN}(1 + r_1) - k_{PN}(1 - r_2) \tag{7}$$

As explained above, normalised reaction rates represent steady-state metabolic fluxes, therefore the values of r1 and r2 are smaller than 1, since in the undisturbed system all enzyme reactions proceed mostly in the forward direction. If r1 and r2 are selected equal this significantly simplifies the relation:

$$r_1 = r_2 = r \tag{8}$$
$$0 = k_{CN}(1+r) - k_{PN}(1+r)$$

Since the factors (1+r) can be found in both terms, the relation yields:

$$0 = k_{CN} - k_{PN} \tag{9}$$

Using the first equation of the system (2) model parameters can be uniquely calculated

$$k_{CN} = \frac{\Phi_I}{1-r} \tag{10}$$
$$k_{PN} = \frac{\Phi_I}{1-r}$$
$$k_{CRN} = \frac{r \cdot \Phi_I}{1-r}$$
$$k_{PRN} = \frac{r \cdot \Phi_I}{1-r}$$

By selecting the values for r and Φ_I all the model parameters can be uniquely calculated by using the steady-state equations of the model. Thus it is clear that it is possible to reduce the number of independent model parameters from four to two by assuming that the steady-state backward/forward metabolic flux ratio is smaller than 1 and that it is equal for the complex and the product formation. The description of the rate constants with the parameters r and Φ_I allows more intuitive experimenting with the model. The following equations for the steady-state values of S_N and C_N can be obtained from the steady-state equations:

$$S_N = \frac{1-r^2}{E_N} + r^2 \cdot P_N \tag{11}$$
$$C_N = 1 - r(1 - E_N \cdot P_N)$$

The equations show how the steady-state values of S_N and C_N will change if the new steady-state levels of E_N and P_N are enforced as a consequence of changes in metabolic processes (xenobiotics). Interestingly, the metabolic flux Φ_I has no effect on the steady-state values of the metabolites, which suggests that for the analysis of the steady-state conditions it is not necessary to exactly know the metabolic flux of the metabolic network. However, the backward/forward metabolic flux ratio has some effect on the steady-state values, especially if r is relatively large. From the equations we can see that the normalised concentrations of the substrate are in reciprocal relation to the normalised concentration of the enzyme, the value of r is present with its squared value, which shows that for small values of r the steady-state values of the substrate primarily depend on the concentrations of the enzyme; the effect of the enzyme concentrations on the concentrations of the complex is much smaller. The only problem for a unique calculation of the steady-state values of S_N and C_N represents the usually unknown steady-state value of P_N after perturbation; however, if r is very small the influence of changed levels of P_N is also small.

The whole metabolic network can be described by combining the enzymatic reaction models. If the network consists of a single linear pathway, the metabolic flux through the network in steady-state remains equal to the initial metabolic flux. However, if the network consists of several interconnected pathways the steady-state ratio of the metabolic fluxes at each crossing of the metabolic pathways (branching points) has to be introduced as an additional model parameter. For the tests performed in this study, the value for r was varied and was finally chosen 0.01 for all the reactions in the network while the chosen metabolic flux ratios at branching points are shown in Fig. 1. The branching ratios affect the steady-state of the branching point significantly if the concentrations of one or more enzymes immediately after the branching point are changed, since the flux ratios can change significantly after the perturbation. The metabolic flux at the beginning was set to 100, however, as shown above, its choice has no effect on the steady-state values of metabolite concentrations. In any metabolic network, there are also irreversible steps, at the latest, when a metabolite is eliminated from an organism. This solves the problem of the unknown value of the steady-state of P_N after perturbation. Since the last step of the network is irreversible, r of the last reaction is equal to 0 and the new steady-states of all the metabolites are uniquely set. Without irreversible reactions the network has an infinite number of possible steady-states.

4 Results

The model was used to predict the results of primary human hepatocites experiments, where liver cells were treated with two different compounds, substance 1 and substance 2. To reproduce the measured levels of metabolites after perturbation with the model enzyme levels were forcefully changed in the model. The results are gathered in Tabs. 1 and 2.

Tab. 1 Simulated and measured metabolite levels relative to normal steady-state values after the treatments with the substances 1 and 2, - means no change

metabolite	substance 1		substance 2	
	sim.	exp.	sim.	exp.
lanosterol	-	-	-	-
FF-MAS	3.2	3.2	0.5	0.5
T-MAS	-	-	-	-
zymosterol	-	-	-	-
lathosterol	2.5	2.5	0.5	0.5
7-dehydro cholesterol	34.6	34.2	-	-
desmosterol	1.8	1.8	0.5	0.5
cholesterol	-	-	-	-

Tab. 2 Simulated enzyme levels relative to normal steady-state levels necessary to achieve measured metabolite levels after the treatments with the substances 1 and 2, - means no change

enzyme	substance 1	substance 2
CYP51	-	0.9
TM7SF2	0.3	1.9
SC4MOL NSDHL HSD17B7	-	0.9
EBP	-	0.9
SC5DL	0.3	2.3
DHCR7	0.04	1.2
DHCR24	0.4	1.7

5 Conclusion

To model steady-state situations with dynamical model it requires less data than description of dynamical properties. With steady-state measurements it is possible to correctly predict a steady-state situation after system perturbation, although the simulation of the transient phenomena is false. The nature of metabolic networks allows some simplifications of the model identification since it operates at permanent non-zero metabolic flow. The non-zero steady-state implies limitations on the model parameters values which narrows the model identification space. If the last chemical reaction in the network is irreversible, the values of all model parameters can be calculated from the known steady-state metabolic flux through the network and forward/reverse reaction flux ratio of each reaction. When only testing the network structure response on different perturbations, the forward/reverse reaction ratio can be fixed at some small value for all the reactions and the metabolic flux can be arbitrary chosen as it only affects the transient phenomena but not the new steady-state levels after the perturbation. However, the selection of the flux value must be such that the model responses are not oscillatory as it introduces unnecessary numerical problems. Flux division ratio at branching points of the network has, however, significant influence on steady-state as any disturbance of the enzymes' functions operating at the branching point changes the flux division ratio. As a consequence, the fluxes through the rest of the network are changed in order to adapt to a new situation and this changes also the consecutive metabolite and enzyme levels.

As described above, an enzyme that takes a metabolite as a substrate has the major effect on the metabolite level. In this sense the enzyme levels of the model were adjusted in order to get similar metabolite levels as were measured after treatments with the substances 1 and 2. In case where substance 1 was used, DHCR7 enzyme levels were reduced to almost 0 while other enzymes that had significantly reduced levels were DHCR14, SC5DL, and DHCR24. In case where substance 2 was used, the same four enzymes were affected, however, in this case, the levels of the enzymes were elevated, and none of the enzymes was so drastically changed as in the case of substance 1. As the substances 1 and 2 have been designed to interact with completely different enzymes it is possible, that all the four enzymes are not directly affected by the substances 1 and 2. Instead, they might trigger the same feedback mechanism that can regulate the levels of all four enzymes. However, as feedback mechanisms is dynamical structure, it cannot be identified from static data. However, dynamical model can be quickly improved if some dynamical data is obtained and the model prediction can be expanded to correctly simulate dynamical properties of the system as well.

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