International Journal of Advanced Computer and Mathematical Sciences
ISSN 2230-9624. Vol4, Issue4, 2013, pp269-276
http://bipublication.com

COMPUTATIONAL MODELING OF BIOCHEMICAL REACTION NETWORK BY KINETICS

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[Received-10/11/2013, Accepted-21/11/2013]

ABSTRACT:

The kinetic characterization of enzymes has diverse value for computational biology. All these approaches are different, and it is becoming important that the appropriate approach be used for the intended purpose. A different approach to the measurement of kinetics of enzyme activity was done.
This offers a basic principles and reasoning for the analysis of metabolic networks. The issues arising in the reconstruction of metabolic networks required for analysis and how they can be circumvented. Subsequently, a more elaborate example network representing Michaelis Menten equation is done. Finally, an overview of applications of this analysis and issues arising while applying methods from metabolic pathway analysis to networks is shown. The central concept in constraint-based modeling: the solution space that is bounded through constraints on fluxes. An overview of the different steps involved in metabolic reconstruction and modeling by pointing to an simple aspect is shown.
Metabolic kinetic analysis is a quantitative method to derive kinetic information from such a system that can be used to determine the control and regulatory structure of the system, and to identify and quantify the interaction of effectors with the system. The principles of the method are described, and the relation with metabolic control analysis is discussed.

Keywords: *computational modeling, metabolic network, enzyme kinetics*

[I] INTRODUCTION

The living cells require energy for all vital processes. This is acquired through metabolism where energy is used to build new cells. Metabolism is the sum of all the process by which cells survive and reproduce. It comprises of two types of reactions, catabolic reactions where conversion of complex compounds to derive energy and building blocks is done and anabolic reactions where the synthesis of complex compounds required in cellular processes is carried out. It is very orderly process involving numerous biochemical reactions that are catalyzed by enzymes [1].

Metabolic networks consist of reactions that convert one type molecules into different molecules. Thus, the concentrations of the

molecules and their rates of change invite special interest. Metabolism can be studied on using enzyme kinetics to explore the dynamic properties of the individual reaction. The network quality of metabolism is studied with stoichiometric analysis with the consideration of synthesis and breakdown of molecules. Metabolic control analysis computes the outcome of perturbations in the network utilizing the dynamics of change in concentration and their integration in the network [2]. The modeling approach for individual biochemical reactions of the network is presented. This approach is applicable to other types of networks.

In this paper fundamental structural and dynamic properties of metabolic networks are discussed. The introduction of stoichiometric simulations of networks and the balance in networks are elaborated. The fundamental essentials of a metabolic network model are the compounds with their concentrations and the reactions processes that change the concentrations of the compounds. These reactions are catalyzed by enzymes in biochemical system.

[II] METABOLISM: BIOCHEMICAL REACTION SYSTEM

Metabolism is the sum of all biochemical reactions that takes place in the cell – organ – organism etc. These biochemical reactions are group into pathways that shares the common metabolites. The pathways accomplish purpose of the living cell so that it can grow and reproduce deriving energy from these biochemical reactions. The catabolic pathways utilize complex compounds from the environment and break them down into simpler compounds thereby releasing energy in the form of ATP. Glycolysis is such an example of a catabolic pathway where cell derive energy from molecules of ATP and NADH metabolizing glucose. The anabolic pathway utilizes the breakdown components or smaller molecules along with energy to synthesize new molecules required by the cell. Amino acid synthesis is an example of anabolic pathways [1].

The biochemical compounds participating in metabolism can be divided into two groups. The molecules those are utilized and synthesized in reactions of the metabolic pathways. They represent the metabolic flux to yield an end product. The other group comprises of biochemical compounds that takes part in the biochemical reactions but remains unchanged or regenerated and utilized in other reactions. Enzymes catalyses these reactions and drive the flux through pathways [2]. ATP is a ubiquitous coenzyme that is being synthesized in catabolic pathways from ADP and a phosphate group but usually is consumed in anabolic pathways leaving a phosphate group and ADP.

[III] MICHAELIS-MENTEN KINETICS

Michaelis–Menten model is one of the simplest and best-known models of enzyme kinetics. The model takes the form of an equation describing the rate of enzymatic reactions, by relating reaction rate to, the concentration of a substrate S [3]. The example of partition of time scales was derived for an irreversible enzyme-catalysed reaction involving single substrate and single product:

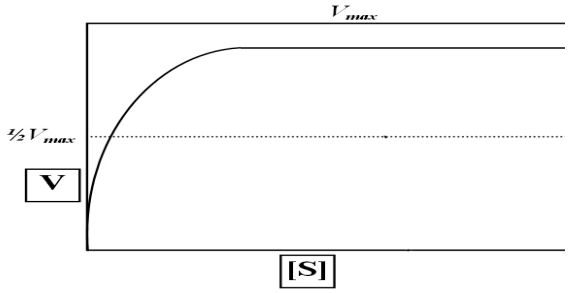

Figure 1: Michaelis-Menten plot. The reaction rate v is plotted as a function of [S], the substrate concentration.

$$S + E \underset{k_3}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_2} E + P \qquad (1)$$

When this system is represented with reaction velocities described by mass action kinetics, it

consist of the four dynamic components *S*, *E*, *ES*, *P*, with the four reaction rates k_1, k_2, k_3 and k_4 and four initial values of the components as parameters. The parameters k_1 and k_3 describe the rate of enzyme-substrate association and dissociation, while k_2 is called the turnover rate of the enzyme. The four dynamical variables reduce to two because of the conservation relations $ET = E + ES$ = constant and $S + P$ = constant.

The beginning of the time scale separation in this system is the statement the reversible binding of the substrate to the enzyme occurs at a much faster rate, described by the rate constants $k1$ and $k3$, than the turnover of the bound substrate and subsequent release of product, described by the rate constant k_2. Thus, in modeling this conversion of substrate *S* to product *P*, the following simplification is made. Because the reversible process is much faster than the irreversible process

$$S + E \underset{k3}{\overset{k1}{\longleftrightarrow}} ES \qquad (2)$$

$$ES \xrightarrow{k_2} E + P \qquad (3)$$

It is assumed that reaction (2) reaches a steady state before reaction (3) has had a noteworthy effect on the system dynamics. This is called the quasi-steady state assumption.

Schematically, after time scale separation the reaction can be represented in the simpler form

$$S \xrightarrow{(ET,Km,k2)} P \qquad (4)$$

Kinetics	Species	Conserved quantities	Parameters	Dimension
MA mass action	*S, E, ES, P*	*S + P*	*k1, k3, k2*	2
MM Michaelis–Menten	*ET = E + ES*	*S, P S + P*	*ET, k2, Km*	1

Table 1: The dimension of model

The corresponding model contains two dynamical variables *S* and *P*, controlled by the conservation *S + P* = constant and two kinetic constants K_M and k_2 and total enzyme concentration *ET* and two initial values for *S* and *P*. The parameter K_M is called the Michaelis-Menten constant. The ratio of the Michaelis-Menten constant *Km* and the substrate concentration *S* determine the enzyme saturation. The biochemical reaction in a metabolic pathway, the total concentration of enzymes becomes a parameter of the reaction kinetics. The time scale partition decreases the quantification of a modeled system (Table 1) and thus saves computational costs.

[IV] STEADY STATE KINETICS

The synthesis and breakdown of metabolite rates are extremely fast and around at the similar level whereas the concentration of a metabolite is at steady state level. It is exhibited that the catalytic action of an enzyme under steady state conditions is especially significant for the elucidation of metabolism [2]. The quantification of steady-state reactions is simple, since the rate of reaction is constant at a steady state level. Steady state levels demonstrate additional comparable properties to metabolic levels. Therefore, it is possible to elucidate the performance or function of the enzyme in the reaction by using parameters. Owing to these attributes, the function of enzymes under various dynamic conditions by changing substrate concentrations, the fate of enzyme can be predicted.

The Michaelis Menten equation forms the fundamental for enzyme kinetics. It can be described by the following kinetic mechanism:

$$E + S \underset{k_3}{\overset{k_1}{\longleftrightarrow}} ES \xrightarrow{k_2} P + E \qquad (1)$$

where, E is enzyme, S is substrate, P is product and ES is enzyme substrate complex. The k1, k2, k3 are the rate constants. In this Michaelis-Menten equation it is proceeds with the assumption that:

The concentration of an enzyme [E] is very less than the substrate[S]; i.e., [E] << [S].

The rate of the reaction is proportional with the initial enzyme concentration; i.e., v / [E]0 .
At sufficiently low substrate concentration [S], V increases linearly with [S].
Basic equation of enzyme kinetics is given by

$$V = \frac{V_{\max}[S]}{K_m + [S]} \quad (5)$$

where K_M is the Michaelis constant:

$$K_m = \frac{k_3 + k_2}{k_1} \quad (6)$$

the Michaelis Menten equation can be derived by relating the kinetic performance of the enzymes. Let us consider that the rate of product (P) formation is equal to the initial rate of reaction.

$$\frac{d[P]}{dt} = v_0 \quad (7)$$

$$v_0 = k_2[ES] \quad (8)$$

This implies that the rate of reaction is equal to the conversion of ES complex to Product and free enzyme.
Considering the above relations hold, then, the mass conservations for enzyme and substrate are:

$$[E]_{total} = [ES] + [E]_{free} \quad (9)$$

$$[S]_0 = [S]_{free} + [ES] + [P]; [S]_0 = [S]_{free} \quad (10)$$

The change of enzyme substrate complex [ES] concentration with respect to time $\frac{d[ES]}{dt}$ is equal to the difference of rate of formation k1[E][S] and rate of consumption $K_3[ES]$ - $k_2[ES]$. The velocity of the reaction remains constant during the initial state of the reaction; thus, we have

$$\frac{d[ES]}{dt} = 0 \quad (11)$$

Thus, the relation is derived as

$$K_3[ES] + k_2[ES] = k_1[E][S] \quad (12)$$

Bringing together all the terms with [ES], then

$$(k_3 + k_2)[ES] = k_1[E][S] \quad (13)$$

Dividing both sides to the k1 and [ES],

$$\frac{(k_3 + k_2)}{k_1} = \frac{[E][S]}{[ES]} \quad (14)$$

Multiplying both sides by [ES] and substituting [E] with collecting [ES] terms together, gives us following relation:

$$(KM + [S])[ES] = [E][S] \quad (15)$$

Lastly dividing both sides by KM + [S], we get

$$[ES] = \frac{[E][S]}{K_M + [S]} \quad (16)$$

In addition to this, v_{max} occurs when [ES] = [E]total. Substituting to get final rate equation

$$v_o = \frac{k_2[E][S]}{K_M + [S]} \quad (17)$$

Let us consider $k_2[E] = v_{max}$ and, herewith, it cam be written as

$$v_0 = \frac{v_{\max}[S]}{K_M + [S]} \quad (18)$$

[V] ENZYME CATALYZED REACTIONS

If a small amount of enzyme is used and all but one substrate is kept constant, then the rate of the enzymatically catalyzed reaction depends on the substrate concentration and initial rate like in the equation [4]. The typical notation of the enzyme catalyzed reaction with one substrate can be given as

$$S + E \underset{k2}{\overset{k1}{\longleftrightarrow}} ES \underset{k4}{\overset{k3}{\longleftrightarrow}} P + E \quad (1)$$

where S is substrate, E is enzyme, ES is enzyme-substrate complex and P is product.

The kinetic equations consist of

$$\frac{d[S]}{dt} = k_2[E] - k_1[E][S] \quad (19)$$

$$\frac{d[E]}{dt} = (k_2 + k3)[ES] - k_1[S] + k_4[P][E] \quad (20)$$

$$\frac{d[P]}{dt} = k_3[ES] - k_4[E][P] \quad (21)$$

with a conservation relation given

$[E] + [ES] = [E]_{total}$

It is clear that the derivative of a substrate with respect to time gives the rate. Thus, the rate is a function of compounds S, enzyme concentrations E and kinetic parameters k. However, the enzyme concentration is hidden in the kinetic constants in the parameter vector k; herewith, thus v as a function of s and P, i.e.

$v = v(s; k)$ (22)

The more general form can be written as

$$\frac{d[S]}{dt} = v_2 - v_1 \quad (23)$$

$$\frac{d[E]}{dt} = (v_2 + v_3) - v_1 - v_4 \quad (24)$$

$$\frac{d[ES]}{dt} = v_1 + v_4 - v_2 - v_3 \quad (25)$$

$$\frac{d[P]}{dt} = v_3 - v_4 \quad (26)$$

with a conservation equation

$[E] + [ES] = [E]_{total:}$ (27)

The form of rate equations is as follows:

$v_1 = k_1[S][E];$ (28)

$v_2 = k_2[ES];$ (29)

$v_3 = k_3[ES];$ (30)

$v_4 = k_4[P][E]:$ (31)

again considering the Equation (1) and the substrate binding and dissociation, as well as the product formation step, lead to the following expression for the time dependence of [ES]:

$$\frac{d[ES]}{dt} = k_1[E][S] - k_2[ES] - k_3[ES] \quad (32)$$

At steady state, the concentration of the intermediate complex, [ES], is constant hence d[ES]/dt = 0. Rearranging this equation and setting $K_m = \frac{k_3 + k_2}{K_1}$, we obtain

[E] = [ES] X KM/[S]. (33)

Furthermore, because the concentration of enzyme is constant, we have [E] = [Et] – [ES]. Equating both, we obtain:

$$v = \frac{d[P]}{dt} = k_2[ES] = k_2[E_t]\frac{[S]}{K_m + [S]} \quad (34)$$

k2 X [Et] is sometimes called the maximal velocity v_{max}. This equation holds true if the concentration of the enzyme-substrate complex stays constant, which in turns implies that the concentration of substrate is in large excess. Plotting the reaction velocity, v, against the substrate concentration, [S], gives a rectangular hyperbolic curve (Fig. 1). The parameter K_M has the unit of a concentration and is of central importance in describing the form of the substrate dependence of the reaction velocity.

Inserting K_M for [S] in above equation, it denotes the substrate concentration at which the reaction speed is half of the limiting velocity. If [S}<< KM, then [S] in the denominator can be disregarded and the reaction becomes linear with regard to S, showing first-order characteristics:

$$[S] << K_m \Rightarrow v \approx \frac{v_{max}}{K_m} \times [S] \quad (35)$$

On the other extreme, for high substrate concentrations, S>> K_m , the reaction speed becomes virtually independent of [S] and tends toward v_{max}.

$$[S] >> K_m \Rightarrow v \approx v_{max} = k_{cat} \times [E_t] \quad (36)$$

Most enzymes catalyzed reactions show similar rate performance so far as they exhibit first or higher order dependencies on the substrate at lower substrate concentrations and tend to a limiting rate depending only on the enzyme concentration when the reactant concentrations are high.

[VI] MODELING BIOCHEMICAL NETWORKS

Modeling the biochemical pathways does not require much more than what has been presented above. The equations for rate of reaction are slightly with complexity. The development of high-throughput technologies and generation vast

amounts of data from diverse levels has necessitated the description, analysis and prediction of models and their processes. The advent of mathematical modeling and computational methodologies has provided the facilities to study the system as a whole including processes at all scales instead of traditional method of focusing on single part of a system [5]. Modeling is a powerful tool in all scientific fields; however, it is always an abstraction and simplification of the real system. The complex biological system like metabolic pathways can be studied through various approaches and modeling of the same system is possible by various ways. This is completely lies on the nature of problem and objectives governing different strategies. So, the question arising from one system may not be solved using similar approach applicable to other systems. Considering a single aspect is explained then minimal models covering limited components thereby making modeling very simple would be sufficient. On the other hand, the account and intense investigation of processes comprising diverse scales and parts requires complex computation and the representation becomes more difficult. Here, attempts were made to stress on models of metabolism able to predict the behavior of single components [6]. In this system, the metabolites and enzymes and the development of higher order process, like pathways and the overlying network were considered.

[VII] KINETIC MODELING OF METABOLIC NETWORK

Metabolism is a dynamical process where mass constantly flows along the metabolic pathways. In a constant environment metabolic change will not change over time. The change in the environment conditions leads to response by cell by adapting the metabolic pathway for growth during a transient phase and will end up in another steady state if no further environmental changes occur [7]. This dynamic nature has to be considered while modeling the metabolism. The fundamental quantities of metabolic models are the concentrations of the biochemical species, basically enzymes and metabolites, and the reaction rates that measure the conversion of biochemical.

[VIII] DETERMINISTIC MODELING

In deterministic modeling, the sequential development of metabolic networks is described by a differential equation system for the state *S* which is the vector of concentrations of the biochemical species. The concentration of each species changes with the reactions. This change is a linear combination, a weighted sum over the respective reaction rates where the weights are the stoichiometric coefficients of the species in those reactions [8]. Thus, the sequential development of the state *S* can be expressed as the product of a matrix and a vector:

$$\frac{\mathrm{dS}}{\mathrm{dt}} = N.v(S, p) \qquad (37)$$

N is the stoichiometric matrix and encodes the structure of the metabolic network. The rows denote the species and the columns the reactions. The entries in a row show the reaction a species is involved in either as a reactant or as a product. The entries in a column, on the other hand, say which species are consumed or produced in a reaction and how many molecules of each are involved. The reaction rates are denoted by *v* and depend on the concentrations of the species *S* and parameters *p*, e.g. reaction constants for the case of mass action and initial concentrations of the biochemical species [9].

[IX] STOICHIOMETRIC SIMULATION

Flux Balance Analysis models the fluxes in a metabolic network. The important components for this analysis are the reaction rate of a certain reaction when the metabolic network is in steady state known as flux [10]. The reaction rate gives

the amount of chemical substrate that is utilized and the amount of product that is synthesized during the reaction. The steady-state corresponds to the state where the concentrations of every metabolite do not change. Stoichiometric matrix contains the stoichiometric constraints for every reaction in terms of each chemical in matrix form. Stoichiometry shows that the number of molecules of the chemicals involved react with each other each during the reaction [7].

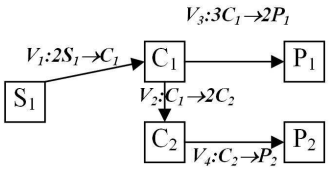

Figure 2: The metabolic network. S1 is substrate, C1 and C2 are chemicals in the network and P1 is product. The reactions are labeled by v where the amount of the input chemical required and synthesis in that reaction.

This information is stated in the stoichiometric matrix, where the columns correspond to all chemicals present in the metabolic network and the rows correspond to all reactions. So for the metabolic network in figure 2, the stoichiometric matrix would look like the matrix in table 2

	V1	**V2**	**V3**	**V4**
S1	-2	0	0	0
C1	1	-1	-3	0
C2	0	2	0	-1
P1	0	0	2	0
P2	0	0	0	1

Table 1: Stoichiometric matrix for the metabolic network.

The determination of the fluxes is made through relating the information from the stoichiometric matrix and assuming that the system is optimized for certain function. This basic assumption in analysis of flux balance is that metabolic networks operate in partial steady state, as such there is no accumulation of species occurs. This can be summarized by the following formula:

Nv = 0 i

where N is the stoichiometric matrix, and v is a vector where vi represents the flow through reaction i.

[X] COMPUTATIONAL MODEL

A model of metabolism comprises several objects on different levels, where partial possible computational representations of is introduced [11]. The focus is given on representation in graph form that offers an easy to understand concept and the most perceptive way to observe the objects of the study, such as molecules, reactions and networks. This graph theory offers many useful tools for their analysis and manipulation[12].

A graph G is a tuple of a set of vertices V and a set of edges E connecting pairs of vertices.

G = (V,E); V = v1, . . . , vn; E μ V × V (38)

A subgraph S of a graph G is the subset of the vertices and edges of G.

S = (VS,ES); VS μ V ; ES μ E (39)

[XI] APPLICATIONS

The results derived from metabolic kinetic analysis can be used in metabolic engineering where one wants to know which parameter should be changed in order to increase the production of some desired chemical. One parameter may be the concentration of some metabolite or enzyme. The most important it gives a deep insight in the understanding the kinetic properties of the enzyme where the actual relations in a reaction network, along with possible feedback loops is not essential [13,14].

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