Հայաստանի Գիտությունների Ազգային Ակադեմիա
Национальная Академия Наук Армении
National Academy of Sciences of Armenia

Հայաստանի Կենսաբանական Հանդես
Биологический Журнал Армении
Biological Journal of Armenia

•Փորձարարական և տեսական հոդվածներ• Экспериментальные и теоретические статьи• •Experimental and Theoretical articles•

Biolog. Journal of Armenia, 2 (61), 2009

COMPUTATIONAL MODELING OF TWO-ENZYME CHAIN IN PRESENCE OF A NON-COMPETITIVE INHIBITOR AND WITH ISOLATED AND COMPLEX INTERACTION BETWEEN ENZYMES

A.G.TAKGYOZYAN

Yerevan State University, E-mail: armen_takgyozyan@yahoo.com

The paper investigates the kinetics of two-enzyme reaction chain both with isolated behaviour and complex interaction between the enzymes, in presence of a non-competitive inhibitor. The comparative analysis of concentration changes of different parameters is presented. The simulation was implemented based on the two models constructed within the *Stella* Dynamic Modelling Package. The models are tested using the same values of initial concentrations for the substrate, enzymes and the inhibitor. The outcomes present several valuable conclusions on the behaviour of the non-competitive inhibitor of isolated and complex interaction of enzymes.

Enzyme – non-competitive inhibitor – complex interaction of enzymes

Աշխատանքում ուսումնասիրվում է երկֆերմենտային ռեակցիայի շղթայի կինետիկան, ֆերմենտների մեկուսացված և կոմպլեքսային փոխազդեցու-թյան պայմաններում, ոչ մրցակցային ինհիբիտորի առկայությամբ: Կատար-վել է տարբեր պարամետրերի կոնցենտրացիաների փոփոխման համեմատա-կան վերլուծություն: Համակարգչային մոդելավորումը իրականացվել է *Stella* դինամիկ մոդելավորման փաթեթով: Մոդելները փորձարկվել են սուբստրա-տի, ֆերմենտների և ինհիբիտորների միևնույն սկզբնական արժեքների ներմուծմամբ: Ներկայացվել են ֆերմենտների կոմպլեքսային փոխազդեցու-թյան առկայության և բացակայության դեպքերում ոչ մրցակցային ինհիբի-տորի վարքը բնութագրող մի քանի եզրակացություններ:

Ֆերմենտ – ոչ մրցակցային ինհիբիտոր - ֆերմենտների կոմպլեքսային փոխազդեցություն

Работа посвящена исследованию кинетики двухферментной цепи в присутствии и отсутствии комплексного взаимодействия ферментов и в присутствии неконкурентного ингибитора. Представлено сравнительный анализ конценрации разных параметров. Компютерное моделирование осуществлено с помощю пакета динамического моделирования *Stella*. Построенные модели апробированы введением одинаковых начальных значений субстрата, ферментов и ингибитора. Сравнительный анализ результатов привел к ряду выводов относительно кинетики неконкурентного ингибирования в присутствии и отсутствии комплексного взаимодействия ферментов.

Фермент – неконкурентный ингибитор – комплексное взаимодействия ферментов

INTRODUCTION

Classical theory of Michaelis-Menten: According to the classical theory of Michaelis-Menten, the enzymatic reaction can be presented as a combination of two parallel reactions.

$$S + E \underset{K2}{\overset{K1}{\rightleftarrows}} ES \qquad\qquad ES \underset{K4}{\overset{K3}{\rightleftarrows}} P + E ,$$

where S is the free concentration of substrate, E is the free concentration of enzyme, ES is the concentration of enzyme-substrate complex, P is the product concentration, K1, K3 and K2, K4 are rate constants for forward and backward reactions respectively [1]. The Michaelis-Menten equation is valid when reaction is in stationary or equilibrium state. In this case the rate of the formation of ES complex equals to the rate of its destruction, i.e. in each instant moment of time the ES concentration is constant. Thus, the Michaelis-Menten equation binds the initial reaction rate V0, maximum reaction rate VMAX and the initial concentration of substrate S.

$$V_0 = \frac{V_{MAX}[S]}{[S] + K_M}$$

The initial reaction rate is defined as the rate of ES complex destruction and equals to

$$V_0 = K_3[ES]$$

KM is the Michaelis constant and is equal to

$$K_M = \frac{K_3 + K_2}{K_1}$$

The physical meaning of KM is that it equals to the substrate concentration when reaction's initial rate is equal to the half of maximum rate (V0=VMAX/2). The kinetics of multi-enzyme systems and systems with different inhibitors is based on the theory of Michaelis-Menten. It also works for each reaction within the enzymatic chains both with isolated enzymes and enzymes in complex interactions.

Non-competitive inhibition: The presence of this type of inhibitor in the enzymatic system does not affect the dissociation constant of the enzyme-substrate complex, instead the maximal reaction rate is reduced [1]. The inhibition that takes place shows that the inhibitor binds to the enzyme, and the stability of the dissociation constant of the enzyme-substrate complex is because the inhibitor and the substrate bind to different structural units of the enzyme. For the non-competitive inhibition it can be theoretically assumed that the enzyme's catalytic and binding centres are not the same. In this case binding to the catalytic centre the inhibitor reduces the enzyme's activity, but does not influence on the formation of the enzyme-substrate complex. It is also possible that the catalytic and binding centres of the enzyme are located in the same place and the inhibitor binds to another structural unit of the enzyme, which changes the latter's conformation and makes it difficult to fulfil the catalytic reaction. For the complete non-competitive reaction the following elementary reactions can be assumed:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P$$

$$E + I \underset{k_5}{\overset{k_4}{\rightleftarrows}} EI$$

$$ES + I \underset{k_7}{\overset{k_6}{\rightleftarrows}} ESI$$

$$EI + S \underset{k_9}{\overset{k_8}{\rightleftarrows}} ESI$$

where ESI is the concentration of the enzyme-substrate-inhibitor complex.

Complex interaction of enzymes: Summarizing several definitions from literature [4,5] it can be stated that enzymes interact in a complex when the activity of one enzyme influences the kinetic parameters of the end product formed by another enzyme in the chain. Thus, complex interactions of enzymes make it impossible to consider them as independent catalysers [5]. There are several types of enzyme-enzyme complex interactions discussed in literature. One of the most common types is called "group transferring chains" [6]. In these structures individual preferential transfer of metabolites occurs from one enzyme of the complex to the other. This phenomenon of transfer is called "metabolic channelling" and is well described in literature [7]. The advantages and disadvantages of the phenomenon are stated in detail in J.Ovadi's work [6], where metabolic channelling is considered as the primary mechanism of enzymes organization.

In the sequential chains of reactions metabolic channelling is represented by preferential transfer of the end-product of one enzymatic reaction to the other enzyme to serve as the substrate of the latter, without interaction with the solution in between [6]. In other words, the molecule of the intermediate product immediately interacts with the next enzyme, and does not add to the concentration of the product itself. Thus, it can be stated that the channelling of metabolites occurs throughout the chain when the step of release of the intermediate product's molecule is skipped [8]. This occurs due to several reasons from which the most important are the ability of complex interaction of participating enzymes and considerable concentration of the intermediate product at the same moment [2, 9].

In case of complex interaction of enzymes in the multi-enzymatic system, the influence of one enzyme on the other is characterised by the global and local parameters of metabolic control analysis [3]. The complex interaction of enzymes can be characterized by following the equation through introduction of an elasticity parameter (denoted as ε):

$$\varepsilon_{E_i}^{\nu} = \frac{\partial \ln \nu}{\partial \ln E_i}$$

where υ is the local rate of the enzymatic reaction, E_i is the concentration of the i^{th} enzyme. The complex interaction of enzymes is characterized by two equations, i.e. the first enzyme's influence on the second's activity and vice versa.

***Materials*.** Simulation of enzymatic chains was organized by STELLA dynamic modeling package [10], where the kinetics of enzymatic reactions is presented by differential equations. The following two models were constructed within the Stella package: (1) isolated two-enzyme chain plus non-competitive inhibitor, (2) two-enzyme chain with complex interaction between enzymes plus non-competitive inhibitor.

Simulation of the models is closely connected with true understanding of an idea of time. Usually the duration of the actual biological reaction does not correspond with the simulation time of the model. Thus, introduction of the notion of "conventional time" becomes necessary for detailed description of the model's steps during the simulation. Simulations were done for 4000 conventional time units. Prior simulation of the constructed models selection of a mathematical method for calculation was implemented. As no high variation of any parameter was expected, the Euler method of integration was used. Initial values of parameters are presented in the Tab. 1 below.

Table 1. Initial values of different parameters for simulation of the constructed models (K1, K3, K5, K7, K9, K10, K11, K12, K13, K14 are rate constants of forward reactions, and K2, K4 ,K6, K8, K10 are rate constants for backward reactions).

Parameter	Unit of mesurement	Reactions in the chain	
		first	second
Substrate concentration	mmol	100	-
Enzyme concentration		10	15
Inhibitor concentration		15	-
K1 local rate coefficient	(sec* mmol)-1	0.0005	
K2 local rate coefficient		0.0006	
K3 local rate coefficient		0.05	
K4 local rate coefficient		0.013	
K5 local rate coefficient		0.0006	
K6 local rate coefficient		0.0007	
K7 local rate coefficient		0.07	
K8 local rate coefficient		0.014	
K9 local rate coefficient		0.005	
K10 local rate coefficient		0.0006	
K11 local rate coefficient		0.0001	
K12 local rate coefficient		0.00008	
K13 local rate coefficient		0.0001	
K14 local rate coefficient		0.00008	

Results and discussion. In two enzyme chain, in case of absence of complex interaction between enzymes, concentration of the first reaction product increases until the 200^{th} conventional time unit, and reaches the value of local maximum. Then it decreases until the 1200^{th} conventional time unit. This can be explained by accumulation of the concentration of the first reaction product (which serves as a substrate for second reaction enzyme) before 200^{th} conventional time unit. After the 1200^{th} conventional time unit concentration of first reaction product starts increasing slowly. At the end of observed time period it accepts value of 4.26 mmol. (Fig. 1).

After the 400^{th} conventional time unit, the concentration of product of the first reaction shows sharp fluctuations, which is a result of competition between enzymes. One can notice that those sharp fluctuations in concentration of the first product appear in specific time period and disappear when there is considerable accumulation of product concentration, which breaks competition between enzymes by transferring main course of chain from one enzyme to another. At the end of observed time period in case of complex interaction between enzymes the concentration of the first reaction is more than in case of isolated enzymes for 23%.

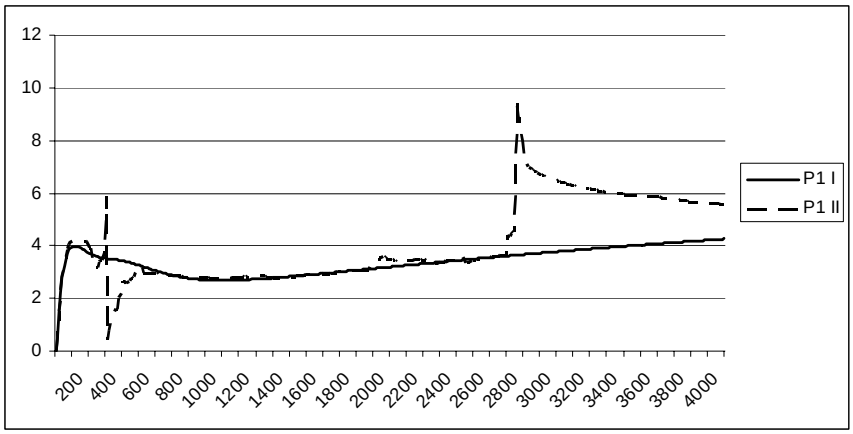

Fig. 1. Dynamics of the first reaction product concentration change, with presence of a non-competitive inhibitor in case of isolated (P1 I) and complex interaction (P1 II) between enzymes.

It is interesting to notice that in case of complex interaction of enzymes, sharp variations in dynamics of the first reaction products concentration are corresponding in that conventional units of time, when concentrations of first reaction enzyme and first reaction enzyme-substrate complex are approximately equal to each other (400^{th} and 2800^{th} conventional time units) (Fig. 2).

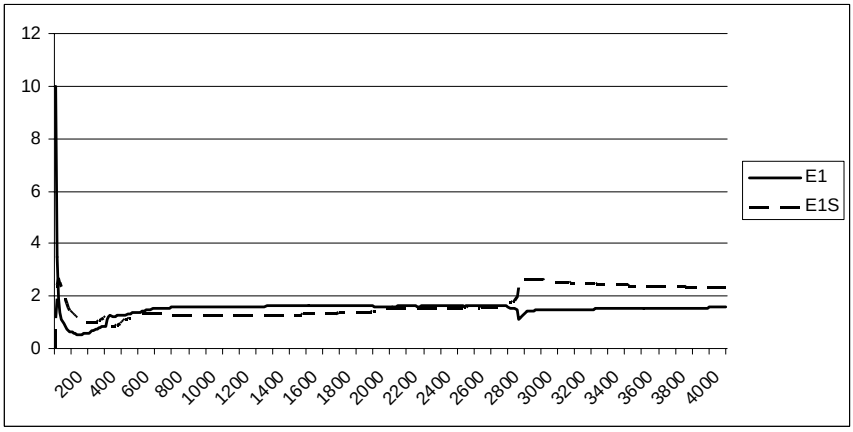

Fig. 2. Dynamics of the first reaction enzyme (E1) and enzyme-substrate complex (E1S) concentrations change, with presence of a non-competitive inhibitor in case of complex interaction between enzymes.

In case of complex interaction of enzymes, concentration of product of the second reaction increases faster than in case of isolated enzymes. There are sharp variations in dynamics of the second product concentration change, which is a result of sharp variations of first reaction product concentration (Fig. 3). Similar to first reaction products, product of second reaction in case of complex interaction of enzymes, is more than in case of isolated enzymes approximately for 23%.

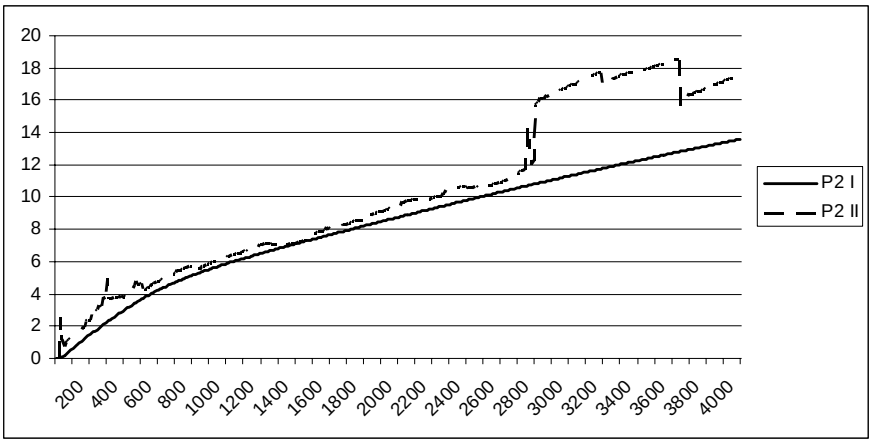

Fig. 3. Dynamics of the second reaction product concentration change, with presence of a non-competitive inhibitor in case of isolated (P1 I) and complex interaction (P1 II) between enzymes

As it shown in figure 4, change dynamics of concentration of inhibitor, in presence and absence of complex interaction between enzymes is approximately the same. In both cases, until 300th conventional time unit it sharply decreases by accepting 6,25 mmol. value of concentration, then increases to approximately 7 mmol. and remains constant until the end of observed time period.

In fact, complex interaction of enzymes brings to addition of end product of the system, but it not affects to dynamics of inhibitor concentration change, i.e. it directly affects to efficiency of enzyme's functioning.

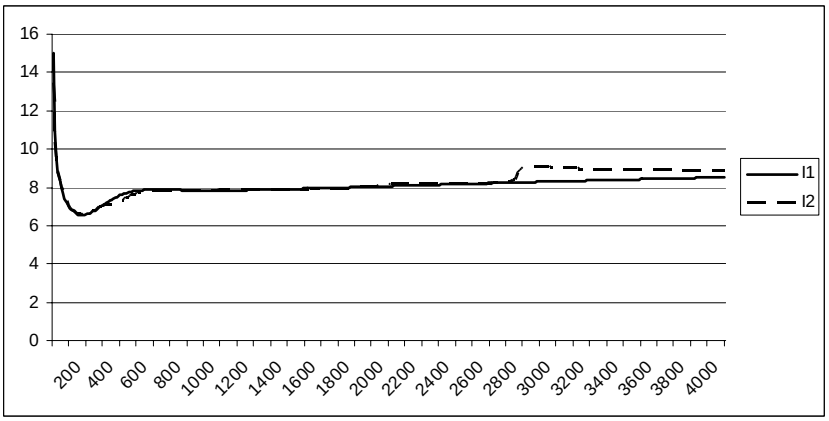

Fig. 4. Dynamics of inhibitor concentration change, with presence of a non-competitive inhibitor and within isolated and complex interaction between enzymes.

CONCLUSIONS

Based on the analysis and comparison of simulation data of computational models the following conclusions were done for the case of two-enzyme reaction chain:

- in case of presence of a non-competitive inhibitor, the complex interaction of enzymes results to the addition of concentrations of products;
- the presence of complex interaction of enzymes does not affect to change dynamics of concentration of inhibitor.
- the presence of complex interaction of enzymes makes sharp variations in concentrations of products due to competition between two enzymes.

REFERENCES

1. *Келети Т.,* Основы ферментативной кинетики. М., Мир, 1990.
2. *Cornish-Bowden A. & Cardenas M.L.,* Control of metabolic processes. New York: Plenum Press,1990.
3. *Takgyozyan A.G., Gevorgyan A.E., Arakelyan V.B., Gevorgyan E.S.,* Electronic J. of Natural Sci., issue 2, 11-17, 2008.
4. *Burns J.A., Cornish-Bowden A., Groen A.K., Heinrich R., Kacser H., Porteous J.W., Rapoport S.M., Rapoport T.A., Stucki J.W., Tager J.M., Wanders R.J.A. & Westerhoff H.V.,* Trends Biochem. Sci., 10: 16-26, 1985.
5. *Sauro H., Kacser H.,* Eur. J. Biochem., 187: 493-499, 1990.
6. *Ovadi J.,* J. Theoret. Biol., *152,* 1-22, 1991.
7. *Mendes P., Kell D.B. & Welch G.R.,* Adv. Mol. Cell Biol., *11,* 1-19, 1995.
8. *Stebbing N., 1980. In: Cell compartmentation and metabolic channeling. / Ed. by Nover L., Lynen F., Mothes K. ,* Amsterdam: Elsevier, 65-105.
9. *Friedrich P.,* Supramolecular enzyme organization: quaternary structure and beyond. – Oxford: Pergamon Press, 1986.
10. *"STELLA Home Page" available at URL: http://www.hps-inc.com/edu/*STELLA

Received 05.03.2009