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COMPUTATIONAL MODELING OF TWO-ENZYME REACTION CHAIN IN PRESENCE OF VARIOUS TYPES OF INHIBITORS

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The paper investigates the behaviour of different types of inhibitors within the two-enzyme reaction chain compared to the kinetics of the chain without any inhibitor present. The simulation was implemented based on four models constructed using "Stella" Dynamic Modeling Package. The models are tested using the same value of initial concentrations of substrate, enzymes and inhibitors and comparative analysis of the concentration changes of parameters are presented. The paper draws several valuable conclusions on the behaviour of competitive, incompetitive and non-competitive inhibitors in the two-enzyme reaction chain.

Enzyme – inhibitor – Michaelis-Menten theory

Աշխատանքում ուսումնասիրվում է տարբեր տիպի ինհիբիտորների վարքը երկֆերմենտային ռեակցիաների շղթայում՝ համեմատելով այն ինհիբիտոր չպարունակող շղթայի վարքի հետ: Համակարգչային մոդելավորումը իրականացվել է "Stella" դինամիկ մոդելավորման փաթեթով: Մոդելները փորձարկվել են սուբստրատի, ֆերմենտների և ինհիբիտորների միևնույն սկզբնական արժեքների ներմուծմամբ: Կատարվել է տարբեր պարամետրերի կոնցենտրացիաների փոփոխման համեմատական վերլուծություն և ներկայացվել են երկֆերմենտային շղթայում մրցակցային, ոչ մրցակցային և անմրցակցային ինհիբիտորների վարքը բնութագրող մի քանի եզրակացություններ:

Ֆերմենտ - ինհիբիտոր – Միքայել-Մենթեսի տեսություն

Работа посвящена исследованию кинетики разных видов ингибиторов в двухферментной цепи по сравнению с поведением цепи без наличия ингибитора. Компьютерное моделирование осуществлено с помощью пакета динамического моделирования "Stella". Построенные модели апробированы введением одинаковых начальных значений концентраций субстрата, ферментов и ингибиторов. Сравнительный анализ результатов привел к ряду выводов относительно кинетики конкурентного, неконкурентного и безконкурентного ингибирования в двухферментной цепи реакций.

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INTRODUCTION

Classical theory of Michaelis-Menten: According to the classical Michaelis-Menten theory, 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 a free concentration of substrate, E is a free concentration of enzyme, ES is a concentration of enzyme-substrate complex, P is a product concentration, K_1, K_3 and K_2, K_4 are rate constants for forward and backward reactions respectively [2]. 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 V_0, maximum reaction rate V_{MAX} 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 [3] and equals to

$$V_0 = K_3[ES]$$

K_M is Michaelis constant and equals to $K_M = \dfrac{K_3 + K_2}{K_1}$

The physical meaning of K_M is that it equals to the substrate concentration when reaction's initial rate is equal to the half of maximum rate ($V_0=V_{MAX}/2$). Michaelis-Menten equation is also usable for each reaction within the enzymatic chains both with isolated enzymes and enzymes in complex interactions. The theory of Michaelis-Menten is basic mechanism for functioning of multi-enzyme systems and systems with presence of inhibitors.

Types of Inhibition: There are various substances which able to inhibit activity of enzymes by interacting with it by different mechanisms. According to the classical conception, the inhibition can be competitive, non competitive, in-competitive and of mixed type. In this paper three types of inhibition, namely purely competitive, purely non competitive and purely in-competitive inhibitions, are discussed [1, 2, 3, 5, 7].

Competitive inhibition: Competitive inhibitors are substrate-alike substances with similar chemical structure [2]. That is why the substrate and the inhibitor compete for the enzyme's active centre. It has been shown that there are competitive inhibitors absolutely differing with their structure from the substrate, but still they bind to the same centres of the enzyme as the substrate does. In case of a purely competitive inhibition the enzyme's all substrate binding centres are able to bind the inhibitor, hence in a presence of high

concentrations of the inhibitor the enzyme's activity will be completely inhibited. Theoretically there can be two possible mechanisms for competitive inhibition. According to the first one, the binding and catalytic centres of the enzyme are overlapping, and the inhibitor that binds to them affects only the binding centre. According to the second version the binding and catalytic centres in the enzyme's molecule are separated and the inhibitor binds to the binding centre. The following reactions can be assumed for the competitive inhibition:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{K_3} E + P$$
$$E + I \underset{k_6}{\overset{k_5}{\rightleftarrows}} EI \quad ,$$

where I is the concentration of free inhibitor and EI – of the enzyme-inhibitor complex.

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 [2]. The inhibitor binds to the enzyme, and the stability of the dissociation constant of the enzyme-substrate complex is due to the fact that 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 by 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:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} E + P$$
$$E + I \underset{k_5}{\overset{k_4}{\rightleftarrows}} EI$$
$$ES + I \underset{k_7}{\overset{k_6}{\rightleftarrows}} ESI \quad ,$$
$$EI + S \underset{k_9}{\overset{k_8}{\rightleftarrows}} ESI$$

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

In-competitive inhibition: The main characteristic of this type of inhibition is that the inhibitor is not able to bind to the enzyme, but it binds to the enzyme-substrate complex. In the purely in-competitive inhibition the enzyme-substrate-inhibitor complex is completely inactive and does not form an end product.

The following elementary reactions can be assumed for the complete in-competitive inhibition:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \underset{k_4}{\overset{k_3}{\rightleftarrows}} E + P$$

$$ES + I \underset{k6}{\overset{k5}{\rightleftarrows}} ESI$$

The in-competitive inhibitor is in interaction with the enzyme, which can be characterized with the following equation:

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

where $C_{E_i}^{\nu}$ is the flux constant of the enzyme, ν is the chain flow and the E_i is the concentration of the enzyme being inhibited [8]. This equation is very similar to the equation characterizing the enzymes' interaction in complex [4, 6]. However, this type of inhibition is very rarely described for biological systems. The only example found in literature was the in-competitive inhibition of alcalinephosphate by L-phenilalanin [9].

Methods. Simulation of enzymatic chains was implemented in the *"Stella"* dynamic modeling package [10]. The following four models were constructed within the Stella package:

(1) Isolated two-enzyme chain based purely on Michaelis-Menten theory;
(2) Isolated two-enzyme chain plus in-competitive inhibitor (I);
(3) Isolated two-enzyme chain plus competitive inhibitor; and
(4) isolated two-enzyme chain plus non-competitive inhibitor.

The simulation of models is closely connected with true understanding of an idea of time since the duration of 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. As no high variation of any parameter was expected, the Euler method of integration was used as mathematical method for calculation. The initial values of parameters used in simulation are presented in Table 1 below.

Results and Discussion. The results of the models' simulation using the same initial values of parameters and comparative analysis of the concentration changes of the substrate for various cases are shown in Figure 1 below.

As one can notice from the figure, the presence of an in-competitive inhibitor sharply decreases the use of substrate. At the end of the observed time period the main part of substrate concentration (87.7%) continue to exist in environment, while in case of absence of any inhibitor there is just 10% of substrate concentration in environment at the end of simulation. The enzyme-substrate complex is forming in both of cases. In presence of an in-competitive inhibitor enzyme-substrate complex binds to inhibitor, forms enzyme-substrate-inhibitor complex which is absolutely inactive and does not produce any

product. In fact the main part of enzyme is used in formation of enzyme-substrate-inhibitor complex and it can not achieve its main function which is the transformation of substrate to product. This leads to the main part of substrate concentration to remain free in environment.

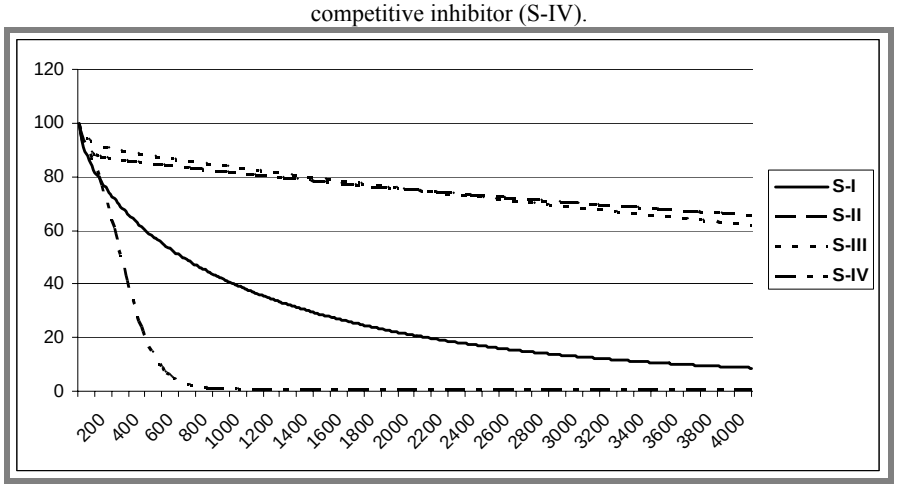
Table 1. Initial values of different parameters for simulation of the constructed models (K1, K3, K5, K7, K9, K11, K13 are rate constants of forward reactions, and K2, K4 ,K6, K8, K10, K12, K14 are rate constants for backward reactions).

Parameter	Unit of measurement	Reactions in the chain	
		first	second
Substrate concentration	mmol	100	-
Enzyme concentration		10	15
Inhibitor concentration		15	-
K_1 local rate coefficient	(sec* mmol)-1	0.0005	
K_2 local rate coefficient		0.0006	
K_3 local rate coefficient		0.05	
K_4 local rate coefficient		0.013	
K_5 local rate coefficient		0.0006	
K_6 local rate coefficient		0.0007	
K_7 local rate coefficient		0.07	
K_8 local rate coefficient		0.014	
K_9 local rate coefficient		0.005	
K_{10} local rate coefficient		0.0006	
K_{11} local rate coefficient		0.0001	
K_{12} local rate coefficient		0.00008	
K_{13} local rate coefficient		0.0001	
K_{14} local rate coefficient		0.00008	

The presence of a competitive inhibitor leads to sharp decrease of the use of substrate concentration. In comparison to the case with an in-competitive inhibitor, the substrate concentration usage is going slowly until the 2000^{th} conventional time unit because of variety of substrate containing complexes such as enzyme-substrate, enzyme-substrate-inhibitor. The formation of those complexes resulted to the substrate concentration being greater in the beginning of simulation compared to the case with competitive inhibition, where the enzyme-substrate-inhibitor complex s not being formed. The competitive inhibitor increases the usage of the substrate more than the in-competitive inhibitor up to the 2000^{th} conventional time unit, since in case of competitive inhibition the accumulation of product in virtual environment is more, which, is a result of decrease of substrate free concentration.

The sharpest decrease of free substrate concentration is observed in case of non-competitive inhibition. Decreasing until the 900^{th} conventional time unit it reaches approximately zero value and continues like this until the end of simulation.

Figure 1. Comparative dynamics of free substrates concentrations changes (mmol) in two enzyme chains with absence of an inhibitor (S-I); with presence of an in-competitive inhibitor (S-II), with presence of competitive inhibitor (S-III), and with presence of a non-competitive inhibitor (S-IV).



Thus, in presence of a non-competitive inhibition, before the 1000th conventional time unit, we can see that the use of substrate is more than in case of absence of an inhibitor by 37.1%. In case of non-competitive inhibition, the expense of free substrate concentration reaches to 95.5% at the end of simulation. In this case, the substrate is mainly used in formation of substrate containing complexes. Nevertheless, unlike the in-competitive inhibition, the enzyme-substrate-inhibitor complex forms by two ways, through binding enzyme-substrate complex to the inhibitor, or through binding enzyme-inhibitor complex to the substrate. At the end of simulation, the values of free substrate concentrations in chains with in-competitive and competitive inhibitors are very close to each other. Free substrate concentrations in chains with non competitive inhibition and without inhibition is also very close to each other because in both cases there is a rather big expense of substrate, but only in case of inhibition absence the substrate is used expends for accumulation of product.

The concentration of the first product in absence of an inhibitor increases and accepts maximum value at the 2000th conventional time unit, after which it gradually decreases. The decrease is due to formation of the second enzyme-first product complex. At the end of simulation, concentration of first product is the biggest in the case of absence of any inhibitor, because there are no obstacles for enzymes for direct performance of their functions (Fig 2).

In presence of three different inhibitors, concentrations of the first product sharply increase until the 200th conventional time unit (similarly to the case with absence of inhibitor). This event has following biological explanation: in virtual environment there is still some concentration of free enzyme until the mentioned time moment, which the sharply decreases, due to formation of enzyme-containing complexes. After the 200th conventional time unit, concentrations of the first product slowly increase, due to destruction of inhibitor-containing complexes. It is important to note, that when observed period of time extends to 4000 conventional time units, the concentration of the first product of inhibitor

containing systems, increases to a definite maximum, and then gradually decreases by giving advantage to the second products (Fig. 2).

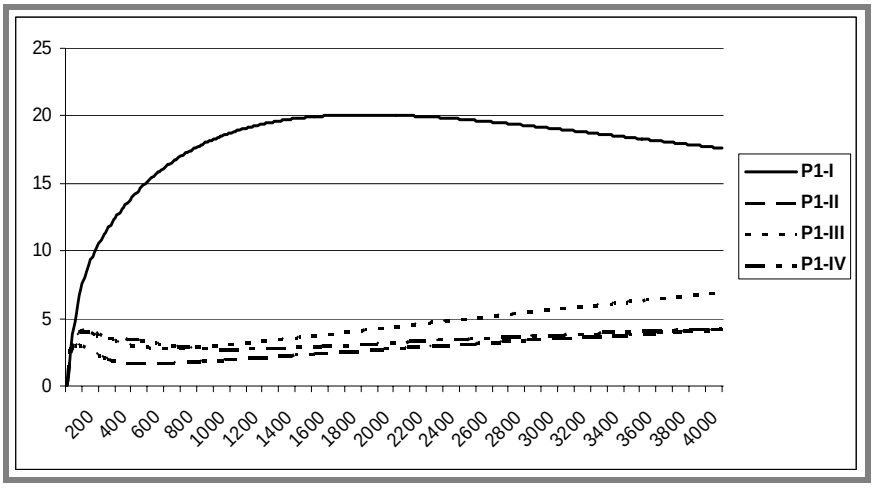


Figure 2. Comparative dynamics of the first product concentrations changes (mmol) in two enzyme chains with absence of an inhibitor (S-I); with presence of an in-competitive inhibitor (S-II), with presence of competitive inhibitor (S-III), and with presence of a non-competitive inhibitor (S-IV).

The dynamics of the second product concentration changes is different from that of first product. First of all, in this case in all four different models, the second product concentration is approximately zero until the 200th conventional time unit due to almost zero concentration of the second reaction substrates, i.e. products of the first reaction (Fig. 3).

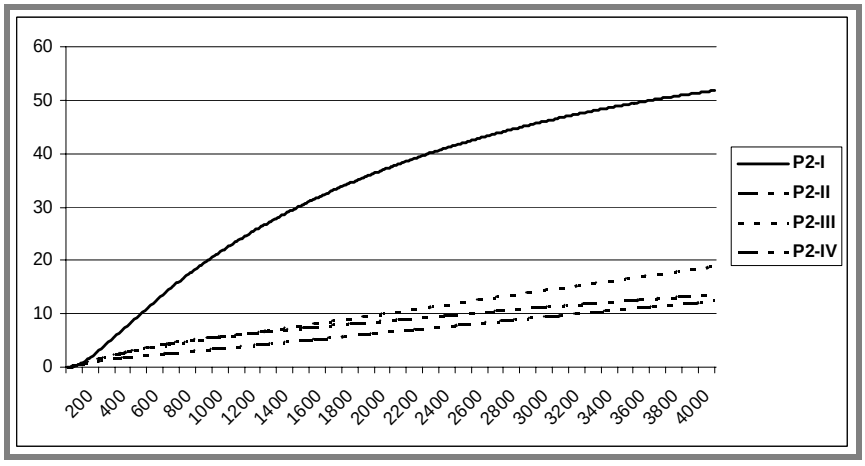


Figure 3. Comparative dynamics of the second product concentrations changes (mmol) in two enzyme chains with absence of an inhibitor (S-I); with presence of an in-competitive inhibitor (S-II), with presence of competitive inhibitor (S-III), and with presence of a non-competitive inhibitor (S-IV).

By observing the final values of the second product at the end of simulation, one can make conclusions on the efficiency of different inhibitors (Table 2).

Table 2. Values of second product concentrations before 2000^{th} and 4000^{th} conventional time units and the efficiency of inhibition in case of various inhibitors.

	before 2000^{th} conventional unit (mmol)	before 4000^{th} conventional unit (mmol)	efficiency of inhibitor (%)
P_2	37.5	51.8	-
P_2 in-competitive	6.6	12.3	76.2
P_2 competitive	10.1	18.8	63.7
P_2 non-competitive	8.7	13.6	73.7

The efficiency of the inhibitor is defined as the percent of decrease of the final product concentrations in enzymatic chain to that of without use of inhibitor. As one can conclude from the Table 2, the smallest concentration of the second product is in enzymatic system with presence of an in-competitive inhibitor. In this case, at the end of simulation, the concentration of final product decreases by 76% compared to the case of absence of inhibitor. The explanation of this event is as follows: first of all, some interaction between inhibited enzyme and inhibitor occurs, which expresses with an elasticity coefficient, used in computational model. In addition, in presence of in-competitive inhibition, the main part of first enzyme concentration is busy in enzyme-substrate-inhibitor complex, which is inactive.

The concentration of the second product in case of non-competitive inhibition gets smaller value at the enc of simulation compared to one in case of competitive inhibition. This is a reasonable result, because the non-competitive inhibitor leads to formation of various inhibitor-containing complexes, such as enzyme-inhibitor complex and enzyme-substrate-inhibitor complex. In fact, in case of non-competitive inhibition, the main part of the first enzyme concentration is included in inhibitor containing complexes, and therefore the system can not realize its direct function - accumulation of the second product. The biggest value of the second product concentration is in case of competitive inhibition. At the end of simulation it decrease for 63,7% comparable to the case with absence of any inhibitor.

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

- In two enzyme system with presence of different inhibitors the biggest expense of substrate is in presence of non competitive inhibition.
- The smallest concentration of final products of two enzyme systems within different inhibitors is in case of in-competitive inhibition.

The biggest concentration of final products of two enzyme systems within different inhibitors is in case of competitive inhibition.

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