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COMPUTATIONAL MODELING OF KINETICS OF THE BISUBSTRATE ENZYMATIC REACTION WITH PING-PONG MECHANISM

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This paper deals with mathematical and computer modeling of bi-substrate enzymatic reactions with ping-pong mechanism, which play an important role in different biochemical pathways. Two models describing these reactions were designed using different software packages, namely “STELLA” dynamic modeling package and “Mathematica 7”. The simulation of the models with different ratios of local rate constants allowed investigating the behavior of reactions as well as determined some interesting aspects concerning influence of each local rate constants on enzyme kinetics.

Computational modeling – bi-substrate enzymatic reactions – ping-pong mechanism

Աշխատանքում իրականացվել է երկսուբստրատ ֆերմենտային ռեակցիաների մաթեմատիկական և համակարգչային մոդելավորում: Այդպիսի ռեակցիաները հաճախ են հանդիպում և կարևոր դեր են խաղում բազմաթիվ կենսաքիմիական ուղիներում: Աշխատանքում նախագծվել են վերը նշված ռեակցիաները նկարագրող մոդել՝ օգտագործելով "STELLA" և "Mathematica 7" համակարգչային փաթեթները: Արագության հաստատունների տարբեր հարաբերությունների դեպքում մոդելի փորձարկումը թույլ է տվել հետազոտել ռեակցիաների վարքը, ինչպես նաև որոշել յուրաքանչյուր արագության հաստատունի ազդեցությունը ռեակցիայի կինետիկայի վրա:

Համակարգչային մոդելավորում – երկսուբստրատ ֆերմենտային ռեակցիաներ – պինգ-պոնգ մեխանիզմ

Данная работа имеет дело с математическим и компютерным моделированием двухсубстратных ферментативных реакций, протекающих механизмом пинг-понг, которые часто встречаются и играют важную роль в различных биохимических путях. Используя динамические программные пакеты “STELLA” и “Mathematica 7”, были сконструированы модели для двухсубстратных ферментативных реакций с механизмом пинг-понг. Симуляция моделей с различными соотношениями локальных констант скоростей позволили изучить поведение этих реакций, а так же установить некоторые интересные аспекты относительно влияния каждой константы скорости на кинетику ферментативной реакции.

Компьютерное моделирование – двухсубстратные ферментативные реакции – механизм пинг-понг

Enzymatic reactions with participation of two substrates are called bisubstrate enzymatic reactions and are widely spread in different metabolic pathways from simple organisms to highly developed ones [2,7,11]. Thus, in silico investigation of these processes can highlight some useful information of specific features of metabolic pathways.

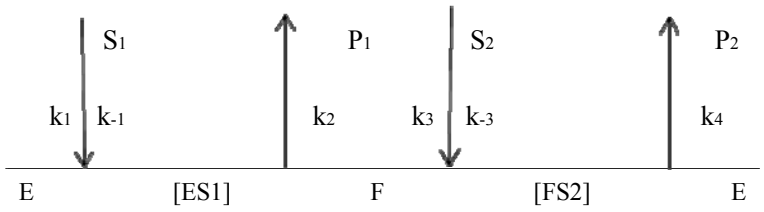
Enzymes almost always catalyze reactions having several substrates, frequently two. Certain enzymes require the presence of a dissociable coenzyme. For kinetic analysis, the coenzyme can be formally considered as a second substrate. Commonly, the concentration of one of the substrates is in large excess and is not significantly modified over the course of the reaction. In the case, when analyzing the kinetics, only the single substrate needs to be taken into account. Enzymatic hydrolysis reactions use water as a second substrate. When those reactions take place in aqueous solution, the second substrate does not contribute to the kinetics of the reaction [10].

There are several well-known mechanisms of bisubstrate enzymatic reactions, namely ping-pong mechanism, sequential mechanism and iso-mechanism [2,4,7,9-11]. These mechanisms differ by order of participation of substrates and by releasing products during enzymatic reaction. In the case of ping-pong mechanism, the product is being already released before all substrates are bound. In the sequential mechanism the two substrates bind before product is released. In iso-mechanisms the enzyme isomerizes into two or more stable conformations.

Ping-pong mechanism of bisubstrate enzymatic reactions.

The ping-pong mechanism can be categorized into two groups, namely random ping-pong and ordered ping-pong mechanisms. The ordered mechanisms are those in which the reaction substrates bind to the enzyme in a defined order. In this work we have examined only ordered ping-pong mechanism.

According to the Cleland's schematic representation of enzymatic reactions, different states of the enzyme can be represented by a horizontal line and the substrates and products by vertical arrows [5]. Thus, the scheme for bisubstrate ping-pong enzymatic reaction is as follows:



where k_1, k_2, k_3 and k_4 are local rate constants of forward reactions, whereas k_{-1} and k_{-3} are local rate constants of reverse reactions; E is concentration of free enzyme; S1 and S2 are concentrations of the first and the second substrates, respectively; [ES1] represents binary complex (E-S_1); [FS_2] is the second binary complex (F-S_2); P_1 and P_2 are the first and the second products of the enzymatic reaction, respectively.

The name of the reaction indicates the alternate binding of substrates and release of products characteristic of this mechanism. After binding of the first substrate, the first product is released, followed by binding of the second substrate and release of the second product. A stringent feature of this mechanism is the formation of an intermediary enzyme form in the reaction with the first substrate, usually by transferring a reactive group. The second substrate will remove this group to the form the second product. Aminotransferase (transaminase) reactions are typical examples of the reactions with ordered ping-pong mechanism.

Considering the general case where the reverse reaction is not negligible, the following rate equation for ordered ping-pong mechanism can be obtained under steady-state conditions [1].

$$v = \frac{k_2 \times E}{1+\frac{K_1}{S_1}+\frac{K_2}{K_4}\left(1+\frac{K_2}{S_2}\right)}$$

where K_1 and K_2 are binding constants of the substrates S_1 and S_2, respectively.

The following system of differential equations describes the bisubstrate ping-pong enzymatic reactions:

$$\begin{cases} \frac{dE}{dt} = k_{-1}[ES_1] + k_4[FS_2] - k_{-1}[E][S_1] \\ \frac{dS_1}{dt} = k_{-1}[ES_1] - k_1[E][S_1] \\ \frac{dES_1}{dt} = k_1[E][S_1] - k_1[ES_1] - k_2[ES_1] \\ \frac{dP_1}{dt} = k_2[ES_1] \\ \frac{dF}{dt} = k_2[ES_1] + k_{-2}[FS_2] - k_2[F][S_2] \\ \frac{dS_2}{dt} = k_{-2}[FS_2] - k_2[F][S_2] \\ \frac{dS_2}{dt} = k_2[F][S_2] - k_{-2}[FS_2] - k_4[FS_2] \\ \frac{dP_2}{dt} = k_4[FS_2] \end{cases}$$

The main objective of this study is to represent the results from in silico investigation of bisubstrate enzymatic reactions with ordered ping-pong mechanism as well as to describe in details the behavior of these reactions. The interests toward such kind of investigation is associated with the lack of experimental data which can be used for running computer models in the field of enzyme biochemistry in general, and in the enzymology in particular. Therefore, in silico investigation of enzymatic reactions can be useful for investigators from both fields.

Materials and methods. In the present work two different modeling software packages are used to design the model of ordered ping-pong mechanisms and to compare results derived from simulation. Modeling has been carried out using "STELLA" dynamic modeling package and "Mathematica 7" software [6, 8] based on the above-presented differential equations. In "STELLA" the computing was done by Euler's method of integration while in "Mathematica 7" the Runge-Kutta's method of integration was used.

One model of ping-pong bisubstrate enzymatic reactions has been constructed for each software package. Since the duration of real biological reactions does not correspond to the model simulation time, the description of kinetic behavior of models has done based on conditional time units (CTUs). The model simulation has been carried out using the following initial values of parameters in both packages:

E_0=10 µmol	$k_1=1.5\times10^{-3}$ (sec×µmol)$^{-1}$	$k_{-1}=1.4\times10^{-3}$ (sec)$^{-1}$
S_1=300 µmol	$k_3=1.5\times10^{-3}$ (sec×µmol)$^{-1}$	$k_{-3}=1.4\times10^{-3}$ (sec)$^{-1}$
S_2=400 µmol	$k_2=3\times10^{-3}$ (sec)$^{-1}$	$k_4=3\times10^{-3}$ (sec)$^{-1}$

From literature it is known, that for aminotransferases which are the typical examples of bisubstrate enzymatic reactions with ping-pong mechanism, binding of substrates is reversible and substrates can be easily dissociate from enzyme-substrate complexes, which means that binding equilibrium constants approximately equal to one. Thus, we have taken appropriate combinations of local rate constants for binding and dissociation of substrates for simulation.

Moreover, the influence of k_2 and k_4 local rate constants on whole kinetics was investigated. Leaving fixed k_1; k_{-1} and k_3; k_{-3} pairs we have examined the cases with the following three ratios of k_2 and k_4: (1) $k_2 = k_4$; (2) $k_2 > k_4$ and (3) $k_2 < k_4$ (refer to Table 1 for values).

Table 1. Different ratios of local rate constants with corresponding values used for simulation

Ratios of local rate constants	Values	
	k_2	k_4
$k_2/k_4=1$	3×10^{-3}	3×10^{-3}
$k_2/k_4>1$	1.5×10^{-2}	3×10^{-3}
$k_2/k_4<1$	3×10^{-3}	1.5×10^{-2}

Results and Discussion. As known, in silico investigation, the computational experiments by different software packages allow identifying some principal features of investigated objects, which are either impossible to determine by in vitro experiments, or they require quite valuable techniques and methodologies. Besides that, computer-based experiment has another advantage, which allows saving time for preparation and implementation of experiments. Taking into consideration the above-mentioned, in this article we have generally concentrated on behavior of enzyme-substrate complexes and intermediary F enzyme form, because usually it is difficult to follow the behavior of these complexes during in vitro experiments.

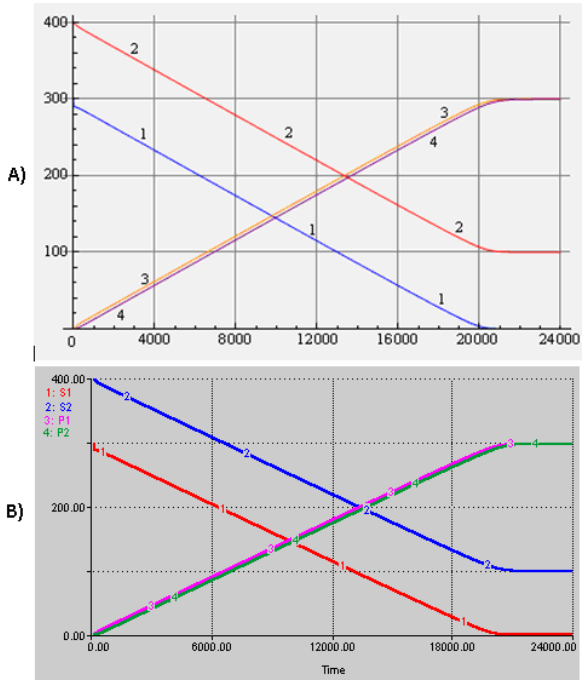


Fig. 1. Kinetic behavior of substrates S_1; S_2 (curves 1 and 2, respectively) and P_1; P_2 (curves 3 and 4, respectively) products in bisubstrate enzymatic reaction with ping-pong mechanism after model's simulation in (A) "STELLA" dynamic modeling package and (B) "Mathematica 7" software.

Simulation in both packages resulted to obtaining the dynamics of bisubstrate enzymatic reactions under bi-bi ping-pong mechanism (fig.1). As one can see from the fig.1, in the very beginning of kinetics the decrease in the concentrations of the first S_1 substrates is

very quick and one possible explanation for such a fast decrease could be that the concentration of the first S_1 substrate have taken with an excess compared to enzyme's concentration ($[E]/[S_1] = 1/30$), and it means that binding of S_1 takes place quite rapidly. The same fast binding of substrates has been previously shown by our group in the case of bisubstrate enzymatic reactions with sequential mechanism [3]. In contrast with the beginning stage, at the final steps of kinetics the decrease in the concentrations of substrates is very slow, and at the end of kinetics, all substrates was completely consumed. Simultaneously, products (P_1 and P_2), in their turn, reach a plateau.

The conditional time unit (CTU) for which the concentration of the [FS2] binary complex approaches to zero is considered end time moment for kinetics (tab. 2).

Table 2. Values of concentrations of various parameters at the end moment of kinetics

Parameters	Values at the end of kinetics in simulation with "STELLA" dynamic modeling package	Values at the end of kinetics in simulation with "Matematica 7" software
E (μmol)	9.89	9.93
S_1 (μmol)	0	0
ES_1 (μmol)	0.02	0.01
P_1 (μmol)	299.98	299.99
F (μmol)	0	0
S_2 (μmol)	100.02	100.01
FS_2 (μmol)	0.09	0.06
P_2 (μmol)	299.88	299.93

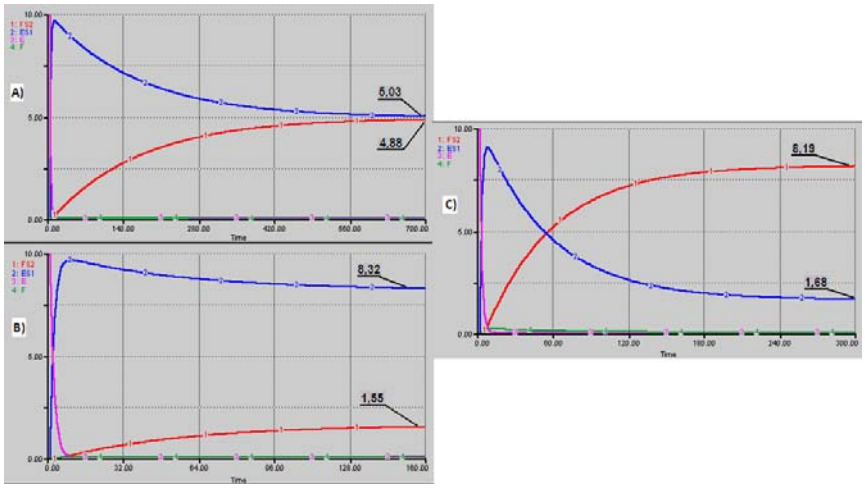


Fig. 2. Pre-steady state kinetics of enzyme-substrate complexes ($[ES_1]$-curve-2; $[FS_2]$-curve-1), free E enzyme (curve-3) and intermediary F form of enzyme ([curve-4) at the following three cases: (A) $k_2=k_4$; (B) $k_2<k_4$ and $k_4/k_2=5$; (C) $k_2>k_4$ and $k_2/k_4=5$

As one can notice from fig. 1 and tab. 2 the simulations under two software show identical results with insignificant differences. Thus, it is more favorable to consider data derived from only one package, which is in our case "STELLA". The simulation emphasized on the study of behavior of enzyme-substrate complexes, free enzyme and intermediary F form of enzyme. To show behavior of enzyme-substrate complexes in more details, we have represented the pre-steady state kinetics (fig. 2) and whole kinetic picture for enzyme-substrate complexes (fig. 3) separately.

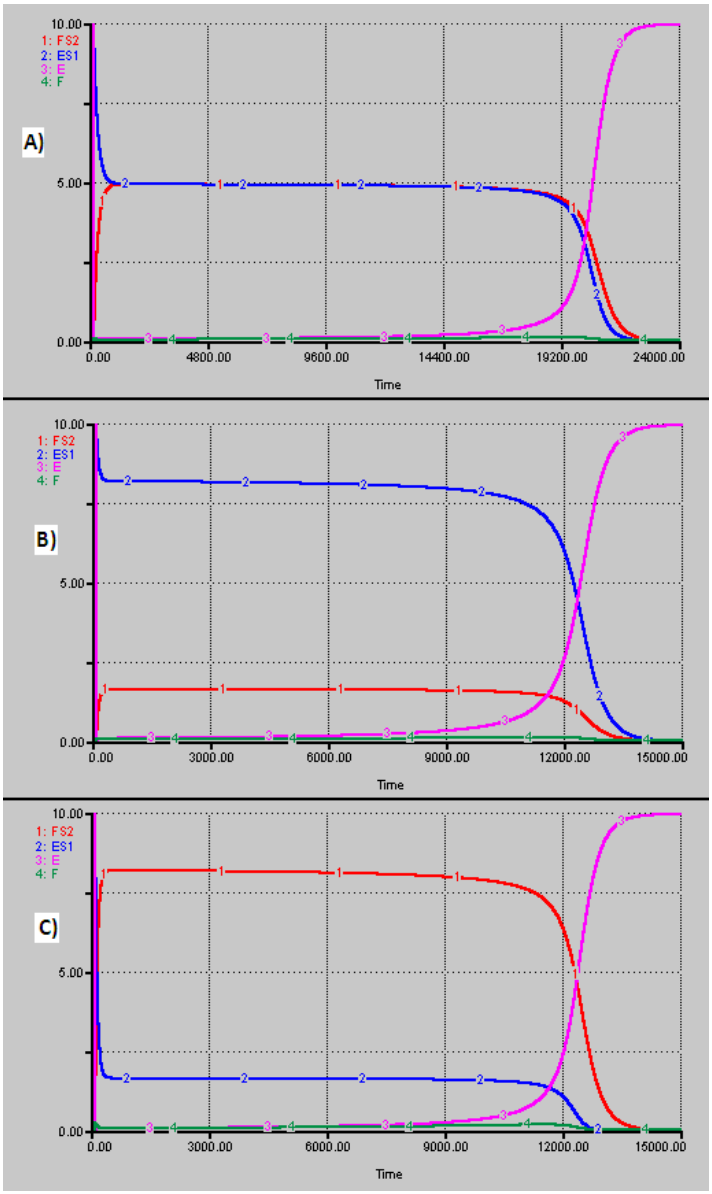


Fig. 3. Overall kinetic behavviour of enzyme-substrate complexes ([ES_1]-curve-2; [FS_2]-curve-1), free E enzyme and intermediary F form of enzyme (curves 3 and 4, respectively) at three different cases: A) $k_2=k_4$; B) $k_2<k_4$ and $k_4/k_2=5$; C) $k_2>k_4$ and $k_2/k_4=5$

As one can see, fig. 2 represents pre-steady state kinetics of complexes, i.e. when [ES_1] and [FS_2] complexes reach saturation point and do not change over the time. The system needs different time to reach steady state point at different ratios of local rate constants. For the first case, system reach steady state after simulation for a 700 conditional time units (CTU), for second case, it needs 160 CTUs for reaching saturation, and for the last case 300 CTUs (tab. 3).

Table 3. Saturation points of [ES_1] and [FS_2] binary complexes at different cases

Ratios of local rate constants	[ES_1] ×(μmol)	[FS_2] ×(μmol)	CTUs needed for saturation
$k_2/k_4=1$	5.03	4.88	700
$k_2/k_4>1$	8.32	1.55	160
$k_2/k_4<1$	1.68	8.19	300

At the first case, when $k_2=k_4$, simulation shows that the concentration of free enzyme rapidly decreases, because of fast binding of the first S_1 substrate and after a few CTU all molecules of enzyme are occupied by substrates (fig. 2A, curve 3). Increase in the concentration of free enzyme was observed in the final part of kinetics (fig. 3A). Concentration of ES_1 complex (fig. 2A, curve 2), naturally reaches its maximum value at 10^{th} CTU (when free enzyme molecules are absent) and after that point it decreases very slowly till certain point and remains constant. Notable decrease in ES_1 concentration could be seen from 20000^{th} and it totally disappears at 24000^{th} CTU (fig. 3A). The concentration of FS_2 complex starts to raise from 10^{th} CTU till the saturation point ([FS_2]= 4.88), which is almost the same as for the first ES_1 complex. Actually, synchronous behavior of curves 1 and 2 lasts until the system turns to the equilibrium state. Like the case of ES_1 complex, in this case as well, the decrease in concentration is seen from 20000^{th} CTU, while total disappearance of the last occurs a little bit later, than as for ES_1 complex. Interestingly, the concentration of intermediate form of enzyme almost equals to zero, which means that the second S_2 substrate rapidly binds to intermediate F form of enzyme, and it is hard to detect F in virtual solution.

In the next case, when k_2 is greater than k_4 by five-fold, the behavior of considered parameters is the same, except for ES_1 and FS_2 binary complexes. The ES_1 complex, as in the previous case, reaches rapidly its maximum value, followed by slow decrease till steady state point, which is equal to 8.32 but notably greater than in previous case (fig. 2, curve 2). As expected, the FS_2 complex slowly increases till saturation point [FS_2]=1.55, which is notable less than in the previous case.

For the third case ($k_2<k_4$) there are not significant changes with free E enzyme and intermediary F form of it. But in the contrast with the second case, the ES_1 and FS_2 binary complexes exchanged "roles". The ES_1 complex, as usual, reaches its maximum value very fast, but then decrease till saturation point, which appeared to be equal to 1.68, less than in previous. Analogically, one might expect that FS_2 complex grows till steady state point, which will be notable greater than in the previous case. And the expectations were met ([FS_2] = 8.19).

The intermediary F form of enzyme has not been detected in notable quantity at all considered cases during pre-steady state kinetics. This fact gives possibility to conclude that intermediary F form quite rapidly binds with the second substrate molecules and transforms to the FS_2 binary complex. The influence of different local rate constant ratios on saturation points of binary complexes could be estimated from tab. 3.

From the tab. 3. it is clearly seen, that the increase in the k_2 local rate constant leads to increase in saturation point for ES_1 binary complex and accordingly to decrease in saturation point for FS_2 complex, while increase of the k_4 local rate constant leads to increase in saturation point for FS_2 complex, and to decrease in saturation point for ES_1 complex.

Comparing the concentrations of the producr in the three various cases, namely (1) $k_2=k_4$; (2) $k_2<k_4$ and (3) $k_2>k_4$, the comparative dynamics of the change product concentration has been revealed (refer to fig. 4 below).

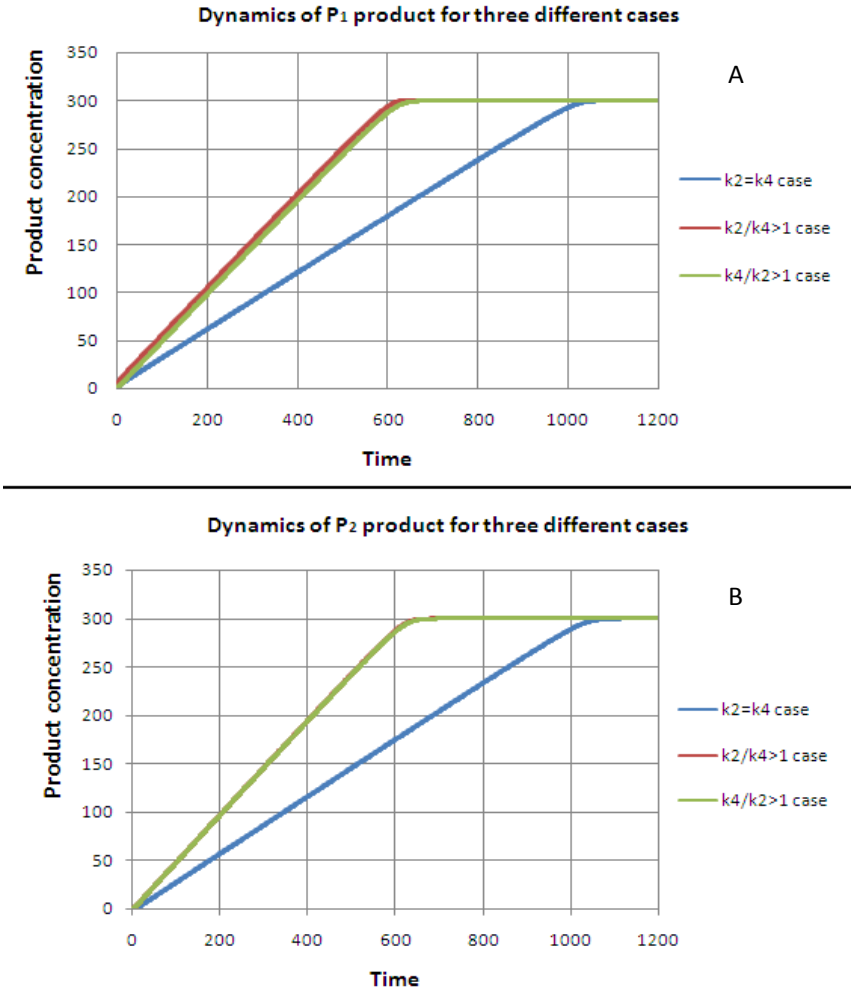


Fig. 4. Dynamics of the change in the concentrations of the first and the second product at three different cases: (1) $k_2=k_4$; (2) $k_2<k_4$; and (3) $k_2>k_4$

As one can see from fig. 4, the increase in the values of local rates in the second and third cases leads to fastening of the whole reaction kinetics, since rates of both first and second product generation were increased. In the second and third cases the increase in the values of local rate constants results to similar behviour of the first product generation dynamics, while the curves of the second produc totally coinside one to another (fig. 4B). Taking into consideration the above-mentioned results one can derive the following conclusions:

1. The computational simulation of models of bisubstrate enzymatic reactions with ping-pong mechanism by "Mathematica 7" and "STELLA" software packages gives identical pictures of kinetics.
2. The interim F form of the enzyme has not been recorded even in virtual environment by modeling using both software packages. Therefore, it can be concluded that F bounds to the second substrate (S_2) very quickly by transforming it into FS_2 complex.

3. In case of equal value of k_2 and k_4 local rate constants the steady-state values of concentrations of the ES_1 and FS_2 complexes are the same.
4. In the case of $k_4>k_2$, the value of ES_1 complex concentration is significantly higher than the value of FS_2 concentration in the steady-state. Similarly, in the case of $k_4<k_2$, the opposite picture occur.
5. The rate of product generation in bi-substrate enzymatic reaction increases regardless the fact which one of k_2 and k_4 local rate constants get biger value.

REFERENCES

1. *Варфоломеев, С.Д., Гуревич, К.Г.* Биокинетика, М., 1999.*Yuan, H., Fu G., Brooks, Ph., Weber, I., Gadda, G.,* Steady-State Kinetic Mechanism and Reductive Half-Reaction of D-Arginine Dehydrogenase from Pseudomonas aeruginosa. Biochemistry, *49*, 9542–9550, 2010.
2. *Келети Т.* Основы ферментативной кинетики, Мир, М., 1990.
3. *Azizyan R.A., Gevorgyan A.E., Arakelyan V.B., Gevorgyan E.S.* Computational modeling of kinetics of the bisubstrate enzymatic reaction with sequential mechanism. Electronic Journal of Natural Sciences, Jan 2011, Issue1, 18, pp. 3-12, 2012.
4. Bisswanger, H. "Enzyme kinetics. Principles and Methods" (2nd edition), WILEY-VCH, 2008.
5. *Cleland, W.W.,* Biochim. Biophys. Acta, *67*, 104–137, 1963.
6. *"Mathematica 7"* Home page available at
7. *Sidhu, R., Blair, H., Human Liver Aldehyde Dehydrogenase.* The J. Biol. Chemistry, *250*, 7899-7904, 1975.
8. *"STELLA Home Page" available at URL: http://www.iseesystems.com/softwares/Education/StellaSoftware.aspx*
9. *Yao C., Lai C., Hsieh H., Chi C., Yin Sh.* Establishment of steady-state metabolism of ethanol in perfused rat liver: the quantitative analysis using kinetic mechanism-based rate equations of alcohol dehydrogenase. Alcohol, *44*, 541-551, 2010.
10. *Yon-Kahn, J., Herve, G.* Molecular and Cellular Enzymology, *1,* Springer, 2010.

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