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Mathematical modeling of Bi-Substrate Enzymatic Reactions with Ping-Pong Mechanism in the Presence of Competitive Inhibitors

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***Abstract*—**The mathematical modeling of different biological processes is usually used to predict or assess behavior of systems in which these processes take place. This study deals with mathematical and computer modeling of bi-substrate enzymatic reactions with ping-pong mechanism, which play an important role in different biochemical pathways. Besides that, three models of competitive inhibition were designed using different software packages. The main objective of this study is to represent the results from in silico investigation of bi-substrate enzymatic reactions with ordered ping-pong mechanism in the presence of competitive inhibitors, as well as to describe in details the inhibition effects. The simulation of the models with certain kinetic parameters allowed investigating the behavior of reactions as well as determined some interesting aspects concerning influence of different cases of competitive inhibition. Simultaneous presence of two inhibitors, competitive to the S_1 and S_2 substrates have been studied. Moreover, we have found the pattern of simultaneous influence of both inhibitors.

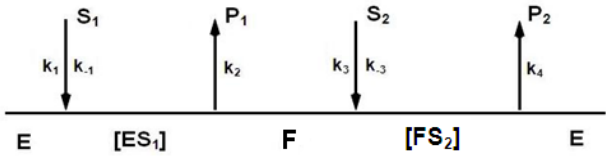
***Keywords*—**Mathematical modeling, bi-substrate enzymatic reactions, ping-pong mechanism, competitive inhibition.

I. Introduction

ENZYMATIC reactions with participation of two substrates are called bi-substrate enzymatic reactions and are widely spread in different metabolic pathways from simple organisms to highly developed ones [1]-[3], [12]. There are several well-known mechanisms of bi-substrate enzymatic reactions, namely sequential mechanism, ping-pong mechanism and iso-mechanism [3], [4], [6].

In the case of ping-pong mechanism product is already released before all substrates are bound. The ping-pong mechanism can be categorized into two groups, namely random ping-pong and ordered ping-pong mechanisms. In contrast to the random mechanism, during the ordered mechanisms substrates bind to the enzyme in a defined order.

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 bi-substrate ping-pong enzymatic reaction is as follows:



where k_1; k_2; k_3; k_4 and k_{-1}; k_{-3} are rate constants of forward and reverse reactions, respectively; E is concentration of free enzyme; S_1 and S_2 are concentrations of the first and the second substrates, respectively; $[ES_1]$ represents binary complex (E-S_1); $[FS_2]$ is for binary complex (F-S_2); P_1 and P_2 are the first and the second products of enzymatic reaction, respectively.

The following system of differential equations describes the bi-substrate ping-pong enzymatic reactions [7], [11]:

$$dE/dt = k_{-1}[ES_1] + k_4[FS_2] - k_1[E][S_1] \quad (1)$$
$$dS_2/dt = k_{-1}[ES_1] - k_1[E][S_1] \quad (2)$$
$$dES_1/dt = k_1[E][S_1] - k_{-1}[ES_1] - k_2[ES_1] \quad (3)$$
$$dP_1/dt = k_2[ES_1] \quad (4)$$
$$dF/dt = k_2[ES_1] + k_{-3}[FS_2] - k_3[F][S_2] \quad (5)$$
$$dS_2/dt = k_{-3}[FS_2] - k_3[F][S_2] \quad (6)$$
$$dFS_2/dt = k_3[F][S_2] - k_{-3}[FS_2] - k_4[FS_2] \quad (7)$$
$$dP_2/dt = k_4[FS_2] \quad (8)$$

An enzyme inhibitor is a compound that binds to an enzyme and interferes with its activity, consequently by slowing down, or in some cases, stopping the catalysis [12]. Cells contain many natural enzyme inhibitors that play important roles in regulating metabolism. Artificial inhibitors are used experimentally to investigate enzyme mechanisms and decipher metabolic pathways. Some drugs, and many poisons, are enzyme inhibitors too [11].

Some inhibitors bind covalently to enzymes causing irreversible inhibition but most biologically relevant inhibition is reversible. Reversible inhibitors are bound to enzymes by the same weak, non-covalent forces that bind substrates and products. Three common types of reversible enzyme inhibition are known in literature: competitive, noncompetitive and uncompetitive inhibition. Here we discuss only competitive inhibition.

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Competitive inhibition occurs when the substrate and an inhibitor resembling the substrate are both added to the enzyme. Once a competitive inhibitor is bound to an enzyme molecule, a substrate molecule cannot bind to that enzyme molecule. Conversely, the binding of substrate to an enzyme molecule prevents the binding of an inhibitor. In other words, S and I compete for binding to the enzyme molecule.

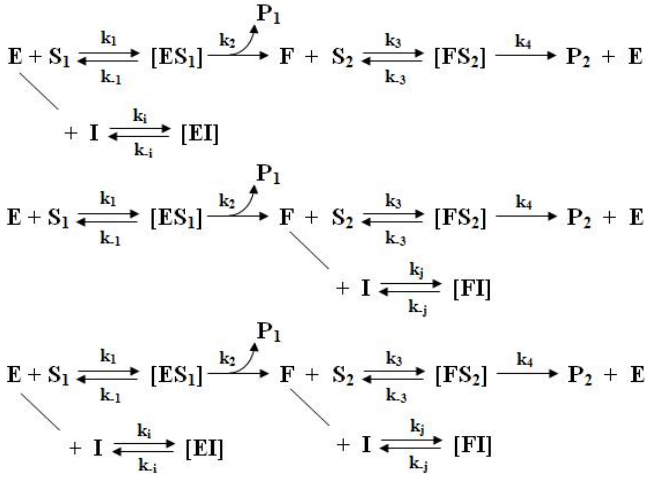
For bi-substrate enzymatic reactions with ping-pong mechanism the following three cases of inhibition are studied:

Competitive inhibition to the first S_1 substrate – designate PPM1 (ping-pong model-1).

Competitive inhibition to the second S_2 substrate – designate PPM2 (ping-pong model-2).

Simultaneous presence of both inhibitors – designate PPM3 (ping-pong model-3).

Schematically, these three cases of inhibition can be represented as follows:



Certainly, for all types of inhibition, differential equations undergo appropriate changes.

II. Methods

Two different modeling software packages are used to design the three models for each above mentioned cases for ordered ping-pong mechanism. Modeling has been carried out using "STELLA" dynamic modeling package and "Mathematica" software based on the above-presented order differential equations (ODEs) [8], [9]. 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.

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.

The following same kinetic parameters are used in all models:

E_0=10 µmol $k_1=2\times10^{-3}$ (sec×µmol)$^{-1}$ $k_{-1}=1\times10^{-3}$ (sec)$^{-1}$
S_1=300 µmol $k_2=5\times10^{-3}$ (sec)$^{-1}$ $k_i=k_j=10^{-3}$ (sec×µmol)$^{-1}$
S_2=300 µmol $k_3=3\times10^{-3}$ (sec×µmol)$^{-1}$ $k_{-3}=1.5\times10^{-3}$ (sec)$^{-1}$
I=30 µmol $k_4=5\times10^{-3}$ (sec)$^{-1}$ $k_{-i}=k_{-j}=7\times10^{-4}$ (sec)$^{-1}$

(4) and (8) correspond to P_1 and P_2 products generation respectively, while substrates consumption determined by (2) and (6).

III. Results and Discussion

The simulation of the above mentioned models by both software packages leaded to similar outcomes. The results of the simulations were discussed below in terms of separate parameters.

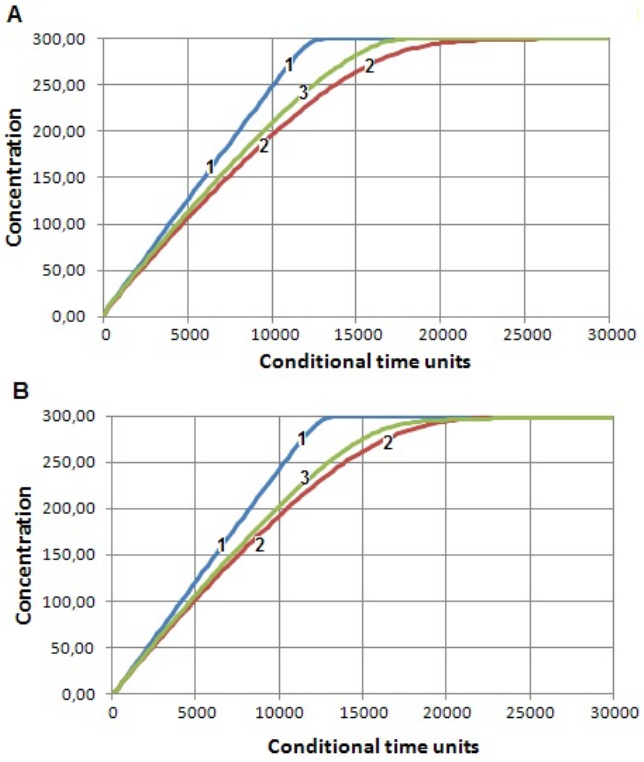


Fig. 1 Concentration changes of the first P_1 (part A) and the second P_2 (part B) products during enzyme kinetics. Curve 1 corresponds to the concentaration changes of P_1 and P_2 from baseline model, without any inhibitors, curve 2 – model with competitive inhibitor to S_1 and curve 3 – competitive to S_2 substrate

It is natural, that dynamics of the change in product concentration shows significant decrease in the rate of product generation in the presence of any inhibitors compared to the baseline case, when no inhibitor is presented (Fig. 1).

As one can notice on Fig. 1, the influence of the competitive inhibitor to the first substrate (curve-2) is a little bit more effective in terms of decreasing the rate of product generation than one to the second substrate (curve-3). This is a result of the fact that the inhibitor to the first substrate binds to the enzyme a little bit earlier than the competitive inhibitor to the second substrate. In other words, the rate of formation $[ES_1]$ and $[FS_2]$ catalytic complexes decrease and the overall enzymatic reaction rate goes down [10].

The more interesting and essential task is the presence of the both inhibitors in virtual solution. The question is, how does this process occurs? What is the law of inhibition effect in the presence of both inhibitors: is it additive, whether it less than in the case of separate presence of inhibitors, or maybe it synergistic?

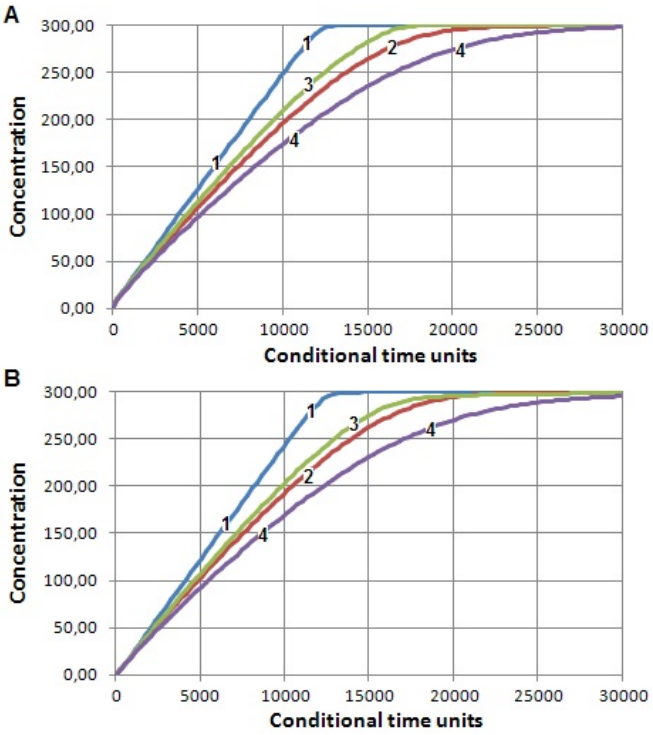


Fig. 2 Concentration changes of the first P_1 (part A) and the second P_2 (part B) products during enzyme kinetics from all considered cases.Curve 1 corresponds to the concentaration changes of P_1 and P_2 from baseline model, without any inhibitors, curve 2 – model with competitive inhibitor to S_1; curve 3 – competitive to S_2 and curve 4 – model with sismultenious presence of both inhibitors

To find answers to the above questions, we have constructed another model (using the same initial kinetic parameters), corresponding to the simultaneous presence of two different competitive inhibitors, for simulation in both software packages. As simulations show, and as it was expected, the inhibition effect of both inhibitors in simulta neous presence is more than inhibition effect of same inhibitors taking separately. This inhibitory effect illustrated in Fig. 2.

From Fig. 2, clearly seen, that curve corresponding to enzyme kinetics in the presence of two different competitive inhibitors (curve-4), lies under the other curves, which means that inhibition effect is the most significant.

For numerical evaluation and comparison of inhibition effects in all studied models, we suggested to represent all derived data corresponding to the time conditional time units (CTUs), when release of products tends to be maximum possible one, in baseline model, where no inhibitor presents. Particularly, that time points correspond to the 1851st, 4035th and 6724th conditional time units, respectively (Table I).

TABLE I
PRODUCT RELEASE IN CONSIDERED FOUR MODELS CORRESPONDING TO THE TIME UNITS OF MAXIMUM POSSIBLE RELEASE OF PRODUCTS DURING ENZYME KINETICS

Models	Values of products concentration (*μmol*) in different models at the end of baseline model kinetics			
	P_1 (at 14280th CTU)	**Consumption (%)**	**P_2** (at 14950th CTU)	**Consumption (%)**
Baseline model	299.99	99.99	299.77	99.92
PPM1	256.56	85.52	261.65	87.21
PPM2	274.64	91.54	274.66	91.55
PPM3	227.60	75.86	230.18	76.72

IV. CONCLUSIONS

The following conclusions can be drawn based on the results of simulations:

In terms of product generation of the bi-substrate enzymatic reaction the competitive inhibitor to the first substrate acts more effectively than the competitive inhibitor to the second substrate.

Simultaneous presence of the both competitive inhibitors influence on the dynamics of product generation with significantly different manner. Moreover, that manner is a little more than additive one.

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REFERENCES

[1] H. Yuan, G. Fu, Ph. Brooks, I. Weber, G. Gadda, Steady-State Kinetic Mechanism and Reductive Half-Reaction of D-Arginine Dehydrogenase from Pseudomonas aeruginosa. Biochemistry, 2010; 49: 9542–9550.
[2] C. Yao, C. Lai, H. Hsieh, C. Chi, Sh. Yin. Establishment of steady-state metabolism of ethanol in perfused rat liver: the quantitative analysis using kinetic mechanism-based rate equations of alcohol dehydrogenase. Alcohol 2010; 44: 541-551.
[3] J. Yon-Kahn, G. Herve. Molecular and Cellular Enzymology. Vol. 1, Springer, 2010.
[4] H. Bisswanger. Enzyme kinetics. Principles and Methods. 2nd ed. WILEY-VCH, 2008.
[5] W. W. Cleland. Biochim. Biophys. Acta 1963; 67: 104–137.
[6] T. Keleti. Basic Enzyme Kinetics. Moscow, «Mir», 1990.
[7] S. D. Varfolomeev, K. G. Gurevich. Biokinetics. Moscow: «FAIR-PRESS», 1999.
[8] "Mathematica 7" Home page available at URL:http://www.wolfram.com/products/mathematica/newin7
[9] "STELLA Home Page" available at URL: http://www.iseesystems.com/softwares/Education/StellaSoftware.aspx
[10] R. A. Azizyan, A. E. Gevorgyan, V. B. Arakelyan, E. S. Gevorgyan. Computational Modeling of Kinetics of the Bisubstrate Enzymatic Reaction With Ping-pong Mechanism. Biological Journal of Armenia, 2 (64), pp. 85-93
[11] C. E. Bugg, W. M. Carson and J. A. Montgomery. Drugs by design. Sci. Am. 1993; 269(6): 92–98.
[12] L. A. Moran, H. R. Horton, K. G. Scrimgeour, M. D. Perry. Principles of Biochemistry. 5th ed. Pearson, 2012.