

Mathematical Modeling of Uncompetitive Inhibition of Bi-Substrate Enzymatic Reactions

Rafayel A. Azizyan, Aram E. Gevorgyan, Valeri B. Arakelyan, Emil S. Gevorgyan

Abstract—Currently, mathematical and computer modeling are widely used in different biological studies to predict or assess behavior of such a complex systems as a biological are. This study deals with mathematical and computer modeling of bi-substrate enzymatic reactions, which play an important role in different biochemical pathways. The main objective of this study is to represent the results from *in silico* investigation of bi-substrate enzymatic reactions in the presence of uncompetitive inhibitors, as well as to describe in details the inhibition effects. Four models of uncompetitive inhibition were designed using different software packages. Particularly, uncompetitive inhibitor to the first $[ES_1]$ and the second $([ES_1S_2]; [FS_2])$ enzyme-substrate complexes have been studied. The simulation, using the same kinetic parameters for all models allowed investigating the behavior of reactions as well as determined some interesting aspects concerning influence of different cases of uncompetitive inhibition. Besides, it has been shown that uncompetitive inhibitors exhibit specific selectivity depending on mechanism of bi-substrate enzymatic reaction.

Keywords—Mathematical modeling, bi-substrate enzymatic reactions, sequential mechanism, ping-pong mechanism, uncompetitive inhibition.

I. INTRODUCTION

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 [1]. Bi-substrate enzymatic reactions are frequent occurrence in metabolic pathways of different organisms [2], [3], and *in silico* studies of these reactions may shed light on some problems of enzyme kinetics and could be helpful to understand mechanisms of bi-substrate enzymatic reactions.

There are several well-known mechanisms of bi-substrate

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enzymatic reactions, namely sequential mechanism, ping-pong mechanism and iso-mechanism [4], [5]. These mechanisms differ by order of participation of substrates and by releasing products during enzymatic reaction. In the case of *sequential mechanism* the two substrates bind before product is released. In the *ping-pong* mechanism, the product is being already released before all substrates are bound. In *iso-mechanisms* the enzyme isomerizes into two or more stable conformations. Here we consider only sequential and ping-pong mechanisms, which are most common mechanisms for bi-substrate enzymatic reactions.

All mentioned mechanisms can be categorized into two groups, namely random and ordered 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 [6]. For instance, in Fig. 1 represented the scheme for bi-substrate enzymatic reaction with sequential mechanism.

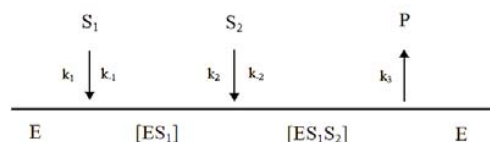


Fig. 1 Scheme for bi-substrate enzymatic reaction with sequential mechanism

where k_1 ; k_2 ; k_3 and k_{-1} ; k_{-2} 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 ($E-S_1$); $[ES_1S_2]$ is for ternary complex; 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 enzymatic reactions with ping-pong mechanism [7], [8]:

$$dE/dt = k_{-1}[ES_1] + k_3[FS_2] - k_1[E][S_1] \quad (1)$$

$$dS_1/dt = k_{-1}[ES_1] - k_1[E][S_1] \quad (2)$$

$$dES_1/dt = k_1[E][S_1] - k_{-1}[ES_1] - k^*[ES_1] \quad (3)$$

$$dP_1/dt = k^*[ES_1] \quad (4)$$

$$dF/dt = k^*[ES_1] + k_{-2}[FS_2] - k_2[F][S_2] \quad (5)$$

$$dS_2/dt = k_{-2}[FS_2] - k_2[F][S_2] \quad (6)$$

$$dFS_2/dt = k_2[F][S_2] - k_{-2}[FS_2] - k_3[FS_2] \quad (7)$$

$$dP_2/dt = k_3[FS_2] \quad (8)$$

The following system of differential equations describes the bi-substrate enzymatic reactions with sequential mechanism [8], [9]:

$$dE/dt = k_{-1}[ES_1] + k_3[ES_1S_2] - k_1[E][S_1] \tag{9}$$

$$dS_1/dt = k_{-1}[ES_1] - k_1[E][S_1] \tag{10}$$

$$dES_1/dt = k_1[E][S_1] + k_{-2}[ES_1S_2] - k_{-1}[ES_1] - k_2[ES_1][S_2] \tag{11}$$

$$dS_2/dt = k_{-2}[ES_1S_2] - k_2[ES_1][S_2] \tag{12}$$

$$dES_1S_2/dt = k_2[ES_1][S_2] - k_{-2}[ES_1S_2] - k_3[ES_1S_2] \tag{13}$$

$$dP/dt = k_3[ES_1S_2] \tag{14}$$

Basically, inhibitor is a compound that binds to an enzyme molecule and interferes with its activity, consequently by slowing down, or in some cases, stopping the catalysis [10]. Inhibitors can act towards preventing the formation of the enzyme-substrate [ES] complex or blocking the chemical reaction that leads to the formation of product. As a general rule, inhibitors are small molecules that bind reversibly to the enzyme they inhibit. Living cells contain many natural enzyme inhibitors that play important roles in regulating metabolism. Artificially synthesized inhibitors are used experimentally to investigate enzyme mechanisms and decipher metabolic pathways. Some drugs, and many poisons, are enzyme inhibitors too [11].

There are also some inhibitors which 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 [12]. Here we discuss only uncompetitive inhibition.

Four cases of uncompetitive inhibition are studied for bi-substrate enzymatic reactions with ping-pong and sequential mechanisms.

Schematically, these four cases of inhibition represented in the following figures (Figs. 2-5).

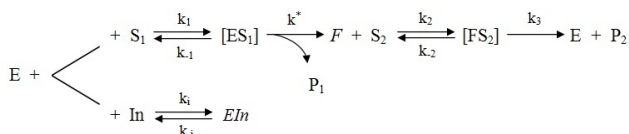


Fig. 2 Uncompetitive inhibition to the first [ES₁] enzyme-substrate complex – designate PPM1 (ping-pong model-1)

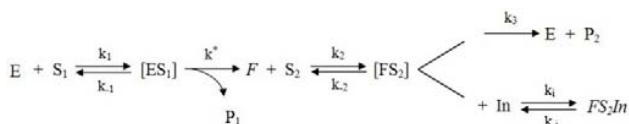


Fig. 3 Uncompetitive inhibition to the second [FS₂] enzyme-substrate complexes – designate PPM2 (ping-pong model-2)

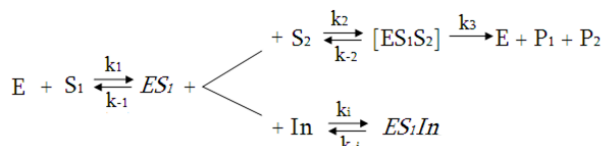


Fig. 4 Uncompetitive inhibition to the first ES₁ enzyme-substrate complex – designate SQM1 (sequential model-1)

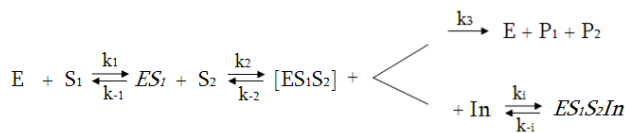


Fig. 5 Uncompetitive inhibition to the second [ES₁S₂] enzyme-substrate complexes – designate SQM2 (sequential model-2)

Certainly, for all inhibition schemes, differential equations undergo appropriate changes.

II. METHODS

The main aim of this study is a comparative analysis of bi-substrate enzymatic reactions with both, sequential, and ping-pong mechanisms in the presence of uncompetitive inhibitors. As it already mentioned, there are several possible cases for uncompetitive inhibition. In this work, we have considered uncompetitive inhibition to [ES₁] binary and [ES₁S₂] ternary complexes for sequential mechanism, as well as uncompetitive inhibition to [ES₁] and [FS₂] binary complexes for ping-pong mechanism. Inhibition analysis has been carried out using different values of inhibitor concentration and has been varied during simulations. Thus, in the first case inhibitor concentration was less than enzyme concentration, in the second – almost equal to the enzyme concentration and in the third one – more than enzyme concentration, while enzyme concentration in all simulated models remains constant.

Three different enzyme/inhibitor ([E]/[I]) ratios

1. [E]/[I]=1/3; ([E]=10 μmol; [I]=30 μmol)
2. [E]/[I]=2/3; ([E]=10 μmol; [I]=15 μmol)
3. [E]/[I]=2; ([E]=10 μmol; [I]=5 μmol)

Two different modeling software packages are used to design four models corresponding to the above mentioned inhibitions as well as two baseline models for ordered ping-pong and ordered sequential mechanisms, without any inhibitor. Modeling has been carried out using “STELLA” dynamic modeling package and “Mathematica” software based on the above-presented order differential equations (ODEs) [13], [14]. In “STELLA” the computing was done by Euler’s method of integration, while in “Mathematica” 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 (CTUs).

The following same kinetic parameters are used in all models:

$$E_0=10 \mu\text{mol} \quad k_1=2 \times 10^{-3} (\text{sec} \times \mu\text{mol})^{-1} \quad k_{-1}=1 \times 10^{-3} (\text{sec})^{-1}$$

$$\begin{aligned}
 S_1 &= 300 \mu\text{mol} & k_3 &= k^* = 5 \times 10^{-3} (\text{sec})^{-1} & k_i &= 10^{-3} (\text{sec} \times \mu\text{mol})^{-1} \\
 S_2 &= 310 \mu\text{mol} & k_2 &= 3 \times 10^{-3} (\text{sec} \times \mu\text{mol})^{-1} & k_{-2} &= 1.5 \times 10^{-3} (\text{sec})^{-1} \\
 I &= 30 \mu\text{mol} & I &= 15 \mu\text{mol} & I &= 5 \mu\text{mol} & k_{-1} &= 7 \times 10^{-4} (\text{sec})^{-1}
 \end{aligned}$$

Equations (4); (8) and (14) correspond to P₁; P₂ and P products generation respectively, while substrates consumption determined by (2); (6) and (10); (12).

To study influence of uncompetitive inhibitor on the bi-substrate enzymatic reactions, we have considered dynamics of products generation. Also, it should be mentioned that for all considered cases time of simulation does not coincide.

III. RESULTS AND DISCUSSION

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

According to the data from simulation of models corresponding to the bi-substrate enzymatic reactions with ping-pong mechanism, in the presence of uncompetitive inhibitor to the first [ES₁] enzyme-substrate complex, concentration changes curves of the first and the second products overlap, while for other mechanisms these curves do not overlap (Fig. 6, curves 3, 4). These fact could be interpreted as that inhibitor binds to the enzyme-substrate complex, thereby prevents quick formation of the first P₁ product and as a result the first and the second products released almost simultaneously.

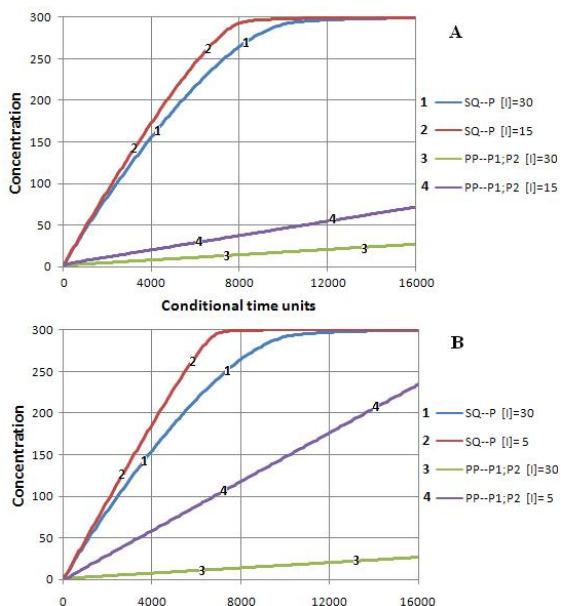


Fig. 6 Comparative dynamics of bi-substrate enzymatic reactions with sequential and ping-pong mechanism, in the presence of uncompetitive inhibitor to the first [ES₁] complex. Inhibitor concentrations are A) 30 μmol and 15 μmol, B) 30 μmol and 5 μmol. Curves: 1,2-product release (sequential mechanism); 3,4-the first product release (ping-pong mechanism); 5,6-the second product release ping-pong mechanism

It is natural, that dynamics of the change in product concentration shows significant decrease in the rate of product generation in parallel with the increase in inhibitor concentration (Figs. 6, 8).

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, for sequential mechanism, with less concentration of uncompetitive inhibitors. Particularly, that time point corresponds to the 8000st conditional time unit (Figs. 7, 9).

As one can notice on Fig. 6, decrease in concentration of uncompetitive inhibitor to the first enzyme-substrate complex, leads to notable increase in product generation for ping-pong mechanism, while for sequential mechanism, product generation almost does not change.

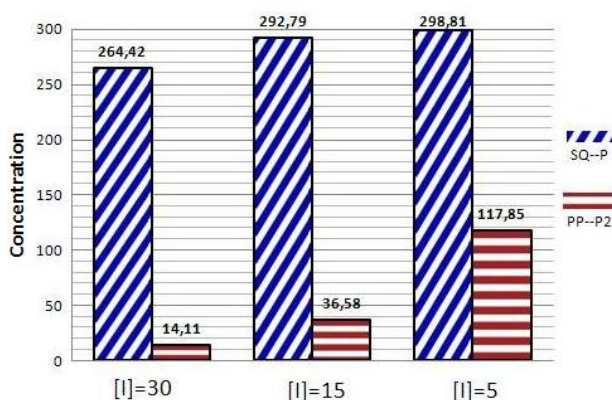


Fig. 7 Product releases of bi-substrate enzymatic reactions with sequential and ping-pong mechanism, in the presence of uncompetitive inhibitor to the first [ES₁] complex. SQ-P - product sequential mechanism; PP-P₂ - the second product ping-pong mechanism. Simulation time is 8000 CTU

Opposite picture of enzyme kinetics take place in virtual solution using uncompetitive inhibitor to the second enzyme-substrate complexes (Fig. 8). Here, increase in concentration of uncompetitive inhibitor influence mainly on the enzymatic reactions with sequential mechanism. Decrease in concentration of inhibitor from 15 μmol to 5 μmol, leads to increase of product generation velocity for sequential mechanism, while for ping-pong mechanism, such a concentration changes of inhibitor do not notable influence on product generation (Fig. 8).

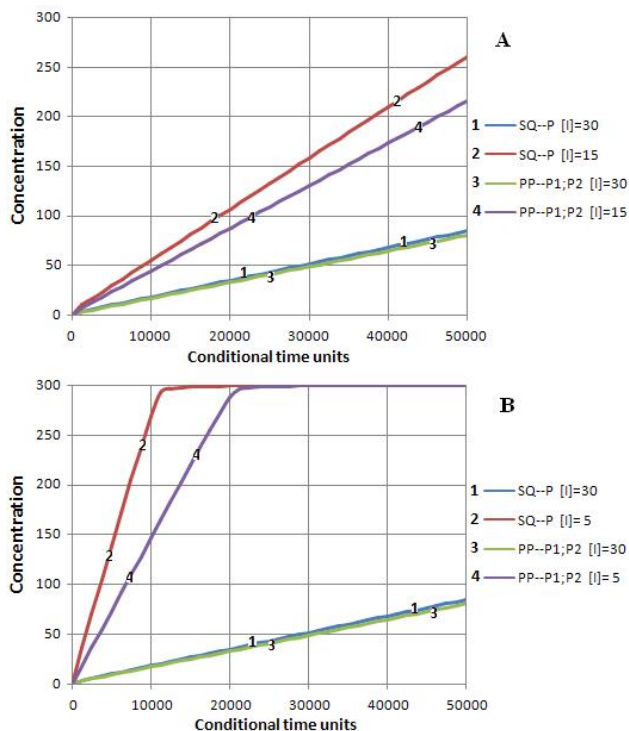


Fig. 8 Comparative dynamics of bi-substrate enzymatic reactions with sequential and ping-pong mechanism, in the presence of uncompetitive inhibitor to the second enzyme-substrate complexes. Inhibitor concentrations are A) 30 μmol and 15 μmol, B) 30 μmol and 5 μmol. Curves: 1,2-product release, sequential mechanism; 3,4-the first product release, ping-pong mechanism; 5,6-the second product release ping-pong mechanism

So, it has been shown that uncompetitive inhibitors exhibit specific selectivity depending on mechanism of bi-substrate enzymatic reaction, which is most interesting results of this study.

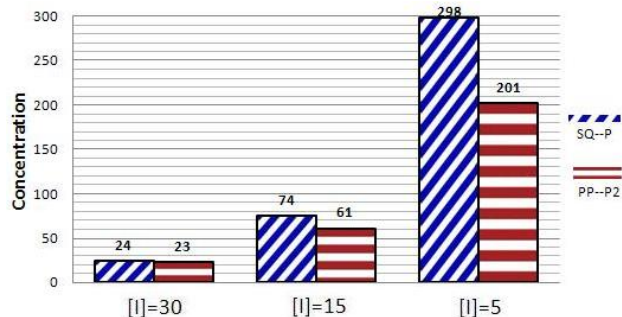


Fig. 9 Product releases bi-substrate enzymatic reactions with sequential and ping-pong mechanism, in the presence of uncompetitive inhibitor to the first $[ES_1]$ complex. SQ-P - product sequential mechanism; PP-P₂ - the second product ping-pong mechanism. Simulation time is 8000 CTU

IV. CONCLUSIONS

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

Mathematical modeling of bi-substrate enzymatic reactions with sequential and ping-pong mechanisms using “STELLA” and “Mathematica” software packages leads to identical kinetic picture.

Uncompetitive inhibitors exhibit specific selectivity depending on mechanism of bi-substrate enzymatic reaction. Thus, in the case of sequential mechanism uncompetitive inhibitor to the second ternary $[ES_1S_2]$ complex exhibit stronger inhibition effect than uncompetitive inhibitor to the first binary $[ES_1]$ complex, while in the case of ping-pong mechanism, uncompetitive inhibitor to the binary $[ES_1]$ complex exhibit stronger inhibition effect than uncompetitive inhibitor to the second $[FS_2]$ complex.

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