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 $([ES_2]; [FS_2])$ 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 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](image)

where $k_1; k_2; k_3$ and $k_{1.2}$ 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$_1$); $[ES_2]$ 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]:

$$
\frac{dE}{dt} = k_{-1}[ES_1] + k_3[FS_2] - k_1[E][S_1] \tag{1}
$$

$$
\frac{dS_1}{dt} = k_{-2}[ES_2] - k_1[E][S_1] \tag{2}
$$

$$
\frac{dES_1}{dt} = k_1[E][S_1] - k_{-1}[ES_1] - k^*[ES_1] \tag{3}
$$

$$
\frac{dP_1}{dt} = k^*[ES_1] \tag{4}
$$

$$
\frac{dE}{dt} = k[E][S_1] - k_{1.2}[ES_2] - k_2[E][S_2] \tag{5}
$$

$$
\frac{dS_2}{dt} = k_{-2}[FS_2] - k_2[F][S_2] \tag{6}
$$

$$
\frac{dS_2}{dt} = k_2[F][S_2] - k_{-2}[FS_2] - k_3[FS_2] \tag{7}
$$

$$
\frac{dP_2}{dt} = k_3[FS_2] \tag{8}
$$

R. A. Azizyan is with Department of Biophysics, Yerevan State University, Yerevan, 0025 Armenia (phone: 00374-94-453037; fax: 00374-10-554641; e-mail: rafayel.azizyan@gmail.com).
A. E. Gevorgyan is with Yerevan State University, Yerevan, 0025, Armenia. (e-mail: aram_gevorgyan@yahoo.com).
V. B. Arakelyan is with Department of Physics, Yerevan State University, Yerevan, 0025, Armenia. (e-mail: v.arakelyan@ysu.am).
E. S. Gevorgyan, is with Department of Biophysics, Yerevan State University, Yerevan, 0025, Armenia. (e-mail: gevorgyan_emil@yahoo.com).
The following system of differential equations describes the bi-substrate enzymatic reactions with sequential mechanism [8], [9]:

\[
dE/dt = k_1[E][S_1] + k_3[E_s][S_2] - k_4[E][S_1] \\
dS_1/dt = k_{-1}[ES_1] - k_1[E][S_1] \\
dES_1/dt = k_5[E][S_1] + k_{-2}[ES_1][S_2] - k_{-1}[ES_1] - k_2[ES_1][S_2] \\
dES_2/dt = k_{-1}[ES_1][S_2] - k_2[ES_1][S_2] \\
d[S_2]/dt = k_2[ES_1][S_2] - k_3[ES_1][S_2] - k_4[ES_1][S_2] \\
d[P]/dt = k_5[ES_1][S_2] \\
\]

Fig. 2 Uncompetitive inhibition to the first \([ES_1]\) enzyme-substrate complex – designate PPM1 (ping-pong model-1)

Fig. 3 Uncompetitive inhibition to the second \([FS_1]\) enzyme-substrate complexes – designate PPM2 (ping-pong model-2)

Fig. 4 Uncompetitive inhibition to the first \([ES_1]\) enzyme-substrate complex – designate SQM1 (sequential model-1)

Fig. 5 Uncompetitive inhibition to the second \([ES_1S_2]\) 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 \([ES_1]\) binary and \([ES_1S_2]\) ternary complexes for sequential mechanism, as well as uncompetitive inhibition to \([ES_1]\) and \([FS_1]\) 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. \([E]/[I]=1/3\); \([E]=10 \, \text{µmol}\); \([I]=30 \, \text{µmol}\)
2. \([E]/[I]=2/3\); \([E]=10 \, \text{µmol}\); \([I]=15 \, \text{µmol}\)
3. \([E]/[I]=2\); \([E]=10 \, \text{µmol}\); \([I]=5 \, \text{µmol}\)

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:

\[E_0=10 \, \text{µmol} \quad k_1=2\times10^{-3} \, (\text{sec}\times\text{µmol})^{-1} \quad k_{-1}=1\times10^{-3} \, (\text{sec})^{-1}\]
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 8000th 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.

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 15µmol to 5µmol, 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. 6 Comparative dynamics of bi-substrate enzymatic reactions with sequential and ping-pong mechanism, in the presence of uncompetitive inhibitor to the first [ES] 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](image1)

![Fig. 7 Product releases of bi-substrate enzymatic reactions with sequential and ping-pong mechanism, in the presence of uncompetitive inhibitor to the first [ES] complex. SQ-P - product sequential mechanism; PP-P₂ - the second product ping-pong mechanism. Simulation time is 8000 CTU](image2)
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 $[ES_2S_2]$ 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.

ACKNOWLEDGMENT

R. A. Azizyan thanks Prof. E. Gevorgyan and V. Arakelyan, as well as Dr. A. Gevorgyan for quite valuable and useful advices during this study.

REFERENCES