REKABETÇİ OLMAYAN ENZİM İNHİBİTÖRLERİ İÇİN MATEMATİKSEL MODEL ANALİZİ

Emrah HASPOLAT
Bilecik Şeyh Edebali University, Faculty of Science, Department of Mathematics, Bilecik
ORCID ID: 0000-0002-1856-3181
Senem YILDIRIM
Bilecik Şeyh Edebali University, Faculty of Science, Department of Mathematics, Bilecik
ORCID ID: 0009-0000-1509-6816

ÖZET

Enzimler, canlı organizmalarda bulunan ve hücresel metabolizmada biyokimyasal reaksiyonları hızlandıran doğal olarak oluşan biyolojik katalizörlerdir. Hücreler, metabolitlerin fizyolojik seviyelerini korumak için çeşitli düzenleyici mekanizmalar kullanırlar. Bu mekanizmalar arasında enzimatik inhibisyon, özellikle rekabetçi olmayan inhibisyon olmak üzere hücresel metabolizmada önemli bir rol oynar ve enzimatik aktivitenin etkili bir şekilde düzenlemesine yardımcı olur.

Matematiksel modelleme, biyokimyasal enzim inhibitörlerinin hedef moleküllerine nasıl bağlandığını anlamaya ve analiz etmeye yardımcı olduğu için sistem biyolojisi alanında çok önemlidir. Bu tür dinamik matematiksel modeller değerli öngörüler sağlar ve doğru sonuçlar üretir. Bununla birlikte, enzimatik rekabetçi olmayan inhibisyonlar matematiksel olarak karmaşık bir kimyasal reaksiyon sistemine sahiptirler. Bu modelleri daha yönetilebilir hale getirmek ve ilgili parametrelerin sayısını azaltmak için belirli teknikler gereklidir. Bu çalışmada, rekabetçi olmayan bir inhibisyon reaksiyonu için dinamik bir model geliştirildi ve modelin dinamik davranışını analiz etmek ve reaksiyon mekanizmasını anlamak için yarı kararlı durum yaklaşımı adı verilen bir model indirgeme tekniği kullanıldı. Bu çalışma sonucunda, indirgenmiş denklem sistemi, yarışmasız inhibitörlerde ürün oluşumu üzerindeki substrat ve inhibitör etkisini analiz etmeyi kolaylaştırmaktadır.

Anahtar Kelimeler: Rekabetçi olmayan inhibisyon, Analitik yaklaşım, Yarı kararlı durum yaklaşımı, Kimyasal reaksiyonlar, Matematiksel model.

AN ANALYSES OF MATHEMATICAL MODEL FOR NONCOMPETITIVE ENZYME INHIBITORS

ABSTRACT

Enzymes are naturally occurring biological catalysts found in living organisms that accelerate biochemical reactions in cellular metabolism. Cells employ various regulatory mechanisms to maintain physiological levels of metabolites. Enzymatic inhibition, notably non-competitive inhibition, is a significant mechanism in cellular metabolism, playing an important role in the regulation of enzymatic activity.

Mathematical modeling is crucial in the field of systems biology as it helps to understand and analyze how biochemical enzyme inhibitors bind to their target molecules. These kinds of dynamic mathematical models provide valuable insights and produce accurate results. However, enzymatic non-competitive inhibitions have a mathematically complex chemical reaction system. Certain techniques are required to make these models more manageable and reduce the number of relevant parameters. In this study, a dynamic model for a non-competitive inhibition reaction is developed and, a model reduction technique, called quasi steady-state approximation, to analyze the dynamic behavior of the model and to understand the reaction mechanism is employed. As a result of this study, the reduced system of equations makes it easier to analyze the substrate and inhibitor effect on product formation in non-competitive inhibitors.

Keywords: Noncompetitive inhibition, Analytical approximation, Quasi steady-state approximation, Chemical reactions, Mathematical model.

1. INTRODUCTION

Enzymes, naturally occurring biological catalysts ubiquitous in living organisms (Bugg 2012; Cornish-Bowden 2012; Frey and Hegeman 2007; Lehninger et al. 2005; Mai et al. 2021; Taylor 2002), facilitate biochemical reactions by interacting with substrates to yield products (Hamad et al. 2023). Despite being effective at low concentrations, enzymes significantly accelerate reaction rates. Every biochemical reaction requires a minimum amount of energy, known as activation energy, irrespective of whether the reaction is exothermic or endothermic. Moreover, enzymes are typically not consumed in reactions but are released alongside the newly formed products upon completion (Bugg 2012; Frey and Hegeman 2007; Mai et al. 2021).

An enzyme inhibitor is a molecule that impedes the catalytic activity of an enzyme (Hamad et al. 2023). These inhibitors are directly involved in the process of catalysis and enzymatic reactions. Enzyme inhibitors are categorized into two primary types: reversible inhibitors and

irreversible inhibitors. Reversible inhibitors transiently bind to enzymes and can be dissociated readily, often by binding to the enzyme's active site to obstruct substrate binding or catalytic action. They further classify into competitive, uncompetitive and mixed (non-competitive) inhibitors (Mohan et al. 2013). Competitive inhibitors, a subtype, resemble the substrate and impede substrate binding by binding to the enzyme's active site. Conversely, uncompetitive inhibitors bind to the enzyme-substrate complex rather than the free enzyme. In non-competitive inhibition, the enzyme possesses two distinct binding sites, which are known as the substrate (known as the active site) and the inhibitor (referred to as the non-competitive site). Non-competitive inhibitors bind to allosteric sites on enzymes, inducing conformational changes that hinder catalytic efficiency, even in the presence of ample substrate concentrations (Berg et al. 2002). When an inhibitor molecule binds to the enzyme, it inactivates the enzyme's active site (Cornish-Bowden 2012; Frey and Hegeman 2007; Taylor 2002).

Mathematical modelling plays a crucial role in the description and analysis of systems in biology, particularly in systems biology. It enables a mathematical understanding of real-world phenomena. Numerous classical studies have focused on modelling biochemical systems using differential equations. Researchers have developed various types of models, including reductions of biochemical reaction networks, approximations of quasi-equilibrium manifolds, quasi-steady-state approximations, reductions in chemical dynamics models, slow invariant manifolds, singular perturbations, and homotopy perturbation method (Akgül et al. 2020; Hamad et al. 2023; Sharmila et al. 2013).

Several procedural steps facilitate the progression of a thought into a theoretical framework and subsequently into a quantitative one. Initially, a theoretical model visually represents the idea through a diagram comprising arrows and boxes. These diagrams aid in deriving mathematical equations that describe the rates of individual processes (Baker 2011; Ingalls 2012; Kot 2001; Lawson and Gleen 2008; Murray 2001; Sontag 2014). The equations extracted from the diagram are then subjected to a process of model reduction.

Chemical reactions can be complex, and it is necessary to understand the basic properties of reaction mechanisms to simplify their complexity (Hamad et al. 2023). Model reduction involves transforming the original system into a simplified version with fewer components (Akgül et al. 2020). In biochemical kinetics, the quasi-steady-state approximation (QSSA) is a prominent method for model reduction and is particularly valuable for addressing the complex dynamics of biochemical reactions. This approach requires the partitioning of the model equations into a subsystem and allows a focused analysis of specific reaction dynamics. This

technique helps to simplify models and facilitate the analysis of their behavior by working with fewer equations. Moreover, it is valuable mathematical tools for simplifying model equations and finding analytical approximate solutions (Akgül et al. 2020).

This study aims to develop and analyse a comprehensive mathematical model for the non-competitive inhibition reactions to explore the effects of substrate and inhibitors on the product formation process.

2. MATHEMATICAL DESCRIPTION OF NON-COMPETITIVE INHIBITION PROCESS

A model diagram with arrows and boxes should be created to represent the idea of a theoretical model. With the help of diagrams mathematical equations can be obtained. Mathematical equations are also used to describe the speed of each process. Equations obtained from the diagram then enter the model reduction process.

2.1. Non-competitive inhibition mechanism and mathematical model

Noncompetitive inhibitors bind to the inactive site on the enzyme and are not attracted to substrates. In this way, substrates and inhibitors can bind to two different points on the enzyme. Thus, the inhibitor disrupts the active site for substrate binding and the three-dimensional structure of the enzyme changes. This reduces the effectiveness of the ES complex.

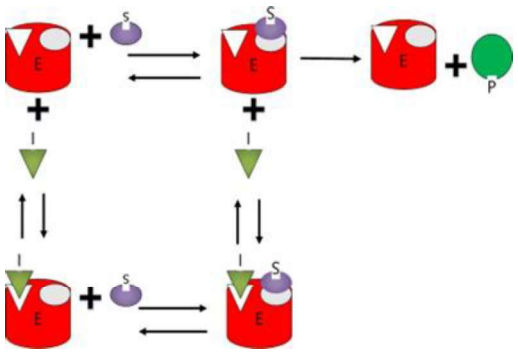

Figure1. Chemical reaction networks for non-competitive inhibitor mechanisms.

The kinetic mechanisms of noncompetitive enzyme inhibitions are provided as follows:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

$$E + I \underset{k_5}{\overset{k_4}{\rightleftharpoons}} EI$$

$$ES + I \underset{k_7}{\overset{k_6}{\rightleftharpoons}} ESI$$

$$EI + S \underset{k_9}{\overset{k_8}{\rightleftharpoons}} ESI$$

where $k_i, \{i = 1, \ldots, 9\}$ are the parameters of the inhibition mechanism. The concentrations of the models are E = [E], S = [S], P = [P], I = [I], A = [ES], B = [EI] and C = [ESI]. The dynamical system of the reaction can be written as follows:

$$dS/dt = -k_1 ES + k_2 A - k_8 BS + k_9 C, \quad (1)$$

$$dE/dt = -k_1 ES + (k_2 + k_3)A - k_4 EI + k_5 B, \quad (2)$$

$$dI/dt = -k_4 EI + k_5 B - k_6 AI + k_7 C, \quad (3)$$

$$dA/dt = k_1 ES - (k_2 + k_3)A - k_6 AI + k_7 C, \quad (4)$$

$$dB/dt = k_4EI - k_5B - k_8BS + k_9C, \quad (5)$$

$$dC/dt = k_6AI - k_7C - k_8BS + k_9C, \quad (6)$$

$$dP/dt = k_3A. \quad (7)$$

Here, the initial conditions of the concentrations in system are, $E(0) = e_0$, $S(0) = s_0$, $I(0) = i_0$, and $A(0) = B(0) = C(0) = P(0) = 0$.

From the conservation laws,

$$\dot{I} + \dot{B} + \dot{C} = 0,$$

$$\dot{S} + \dot{A} + \dot{C} + \dot{P} = 0,$$

$$\dot{A} + \dot{B} + \dot{C} + \dot{E} = 0,$$

which gives the following equations,

$$I + B + C = i_{0,}$$

$$S + P + A + C = s_{0,}$$

$$E + A + B + C = e_{0,}$$

and hence, it can be written as,

$$E = e_0 - i_0 + I - A,$$

$$B = i_0 - s_0 + S + A + P - I,$$

$$C = s_0 - S - A - P.$$

By using the conservation laws and substituting the variables E, B and C into the dynamic system (1-7), the following reduced equations system can be obtained as,

$$dS/dt = -k_1S(e_0 - i_0 + I - A) + k_2A - k_8S(i_0 - s_0 + S + A + P - I) + k_9(s_0 - S - A - P), \quad (8)$$

$$dI/dt = -k_4I(e_0 - i_0 + I - A) + k_5(i_0 - s_0 + S + A + P - I) - k_6AI + k_7(s_0 - S - A - P), \quad (9)$$

$$dA/dt = k_1 S(e_0 - i_0 + I - A) - (k_2 + k_3 + k_6 I)A + k_7(s_0 - S - A - P), \quad (10)$$

$$dP/dt = k_3 A. \quad (11)$$

Let us consider the new dimensionless variables as follows,

$\tau = t$, $u = I/i_0$, $v = S/s_0$, $w = A/e_0$ and $x = P/e_0$.

System within equations (8-11) then turn into the following:

$$dv/d\tau = -k_1 e_0 v(1-w) + (k_1 - k_8) i_0 v(1-u) + (k_8 s_0 v + k_9)[(1-v) - \epsilon(x+w)] - k_2 \epsilon w, \quad (12)$$

$$du/d\tau = -k_4 e_0 u(1-w) + (k_4 i_0 u + k_5)(1-u) + \left(\frac{(k_5 - k_7)e_0}{i_0}\right)(x+w) - \left(\frac{(k_5 - k_7)s_0}{i_0}\right)(1-v) - k_6 e_0 uw, \quad (13)$$

$$\epsilon(dw/d\tau) = [k_1 e_0 v(1-w) - k_1 i_0 v(1-u) + k_7(1-v)] - \epsilon[(k_2 + k_3)w + k_7(x+w) + k_6 i_0 uw], \quad (14)$$

$$dx/d\tau = k_3 w, \quad (15)$$

where the parameter ϵ is $\epsilon = \frac{e_0}{s_0}$.

Since the enzyme concentration is significantly smaller compared to that of the substrate, the $\frac{e_0}{s_0}$ ratio takes a value close to zero. By using QSSA when $\epsilon \longrightarrow 0$, the equation (12-15) in system above turns as follows,

$$dv/d\tau = -k_1 e_0 v(1-w) + (k_1 - k_8) i_0 v(1-u) + (k_8 s_0 v + k_9)(1-v), \quad (16)$$

$$du/d\tau = -k_4 e_0 u(1-w) + (k_4 i_0 u + k_5)(1-u) + \left(\frac{(k_5 - k_7)e_0}{i_0}\right)(x+w) - \left(\frac{(k_5 - k_7)s_0}{i_0}\right)(1-v) - k_6 e_0 uw, \quad (17)$$

$$0 = [k_1 e_0 v(1-w) - k_1 i_0 v(1-u) + k_7(1-v)], \quad (18)$$

$$dx/d\tau = k_3 w. \quad (19)$$

From equation (18), the following equation can be calculated in terms of u and v as follows,

$$w = 1 - \frac{i_0}{e_0}(1-u) + \frac{k_7}{k_1 e_0}\frac{(1-v)}{v}.$$

Then by substituting the variable w into the equations (16, 17 and 19), the equations (16-19) in system above turns as,

$$dv/d\tau = k_8 i_0 v(u-1) + (k_7 + k_9 + k_8 s_0 v)(1-v), \tag{20}$$

$$du/d\tau = \frac{k_7}{k_1}\left[(k_4 - k_6)u + \frac{(k_5 - k_7)}{i_0}\right]\frac{(1-v)}{v} + \frac{(k_5 - k_7)}{i_0}[e_0(x+1) - s_0(1-v)] + k_7(1-u) + k_6 u[e_0 + i_0(1-u)], \tag{21}$$

$$dx/d\tau = k_3\left[1 - \frac{i_0}{e_0}(1-u) + \frac{k_7}{k_1 e_0}\frac{(1-v)}{v}\right]. \tag{22}$$

3. CONCLUSION

This study started by obtaining a system of seven equations derived using a non-competitive diagram. Then, by utilizing conservation laws, the initial seven equations were reduced to four equations. The complexity was further simplified by using the quasi-steady-state approximation (QSSA), resulting in a reduction to three equations. Despite the complexity of the original equations, the reduction of the system to three equations was achieved. With future research, one can investigate the critical points and their stability analysis for comprehensive insights, and potentially numerical methods and phase plane analysis.

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