Kinetics of Enzyme Inhibition

Debjani Sur

Department of Chemistry, Asansol Girls' College, Asansol, West Bengal

Date of Submission: 30th November, 2014 Date of Acceptance 20th December, 2014

Abstract

Enzyme catalyzes virtually every biochemical process. Some kind of regulatory mechanisms exist within the cell to control enzyme catalyzed reactions. Generally the enzymes are modulated in the cell by low molecular weight inhibitors which may or may not be present at all times. Inhibitors also provide a means to study the functional groups at the active sites. For a better understanding of the emerging enzyme inhibitors which includes drugs, insecticides, antibiotics, poison etc. on enzyme mechanism, their study of kinetics is of great importance.

Keywords: Enzyme mechanism, Kinetics, Reversible Inhibition, Competitive Inhibition, Non-competitive Inhibition.

1. Introduction

Enzymes are the reaction catalysts of biological systems which accelerate and direct specific biochemical reactions. For all living organisms, enzymes are necessary for life. It is well known that activities of intracellulary and extracellulary enzymes depend on numerous constituents of medium or circumstances. The most important factors which influence enzyme activity are enzyme concentration, the amount of specific enzyme substrate, electrochemical reaction of medium for enzyme activity (pH), the presence of activators (specific or nonspecific) as well as presence of inhibitors.

Enzyme inhibition is a science of enzyme-substrate reaction influenced by the presence of any

organic chemical or inorganic metal or biosynthetic compound due to their covalent or non-covalent interactions with enzyme active site. It is well known that all these inhibitors follow same rule to interplay in enzyme reaction. The enzyme inhibitors are low molecular weight chemical compounds. They can reduce or completely inhibit the enzyme catalytic activity either reversibly or permanently (irreversibly)[1].

Enzymes are also of extreme medicinal interest as most of the drugs prescribed are enzyme inhibitors. In drug discovery, several drug analogues are chosen and/or designed to inhibit specific enzymes. However, detoxification or reduced toxic effect of many antitoxins is also accomplished mainly due to their enzyme inhibitory action. In this paper, study of enzyme inhibition kinetics is being considered to clarify the mechanisms of enzyme inhibition action and physiological regulation of metabolic enzymes. The enzyme inhibition reactions follow a set of rules. An enzyme interacts and binds with substrate in 1:1 ratio at the active site in the form of a lock-key 3D arrangement for induced fit. Inhibitor active groups compete with substrate active groups and/or active groups at enzyme allosteric catalytic site in a synergistic manner or first come first preference, to make enzyme-inhibitor-substrate/ enzyme-substrate/ enzyme-inhibitor complexes. These complex formations depend on active free energy loss and thermodynamic principles. Intermolecular forces between enzyme subunits, substrate or inhibitor active group interactions, physical properties of binding nature- electrophilic, hydrophilic, nucleophilic and metalloprotein nature, hydrogen bonding affect the overall enzyme reaction rate and mode of inhibition. Inhibition occurs in a variety of ways, but can be broadly classified into two types, reversible and irreversible inhibition.

2. Reversible Inhibition

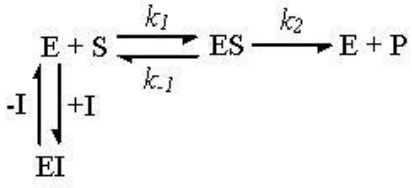
Many inhibitors reversibly binds with the enzyme molecule affecting the equilibrium constant of the reaction. Several types of such inhibitions are known and can be distinguished with the help of Lineweaver Burk plots[2,3] of enzyme kinetics into three sub-types.

a) Competitive Inhibition

The competitive inhibitor is structurally related to the substrate and it competes with the substrate for the active site of the enzyme. It binds reversibly at the active site of the enzyme to form an enzyme-inhibitor (EI) complex. Once the inhibitor occupies the active site of the

enzyme, it prevents binding of substrate to form enzyme-substrate complex (ES) and thus abolishes the formation of normal metabolic product[1,4]. Inhibitor binds reversibly the enzyme and because of that the competition can be decreased simply by adding more substrate.

The equation for enzyme catalyze reaction in presence of competitive inhibitor is as follows



Kinetically, the inhibitor (I) binds the free enzyme reversibly to form enzyme-inhibitor complex (EI) which is catalytically inactive and cannot bind the substrate. The competitive inhibitor reduces the availability of free enzyme for the substrate binding. The total enzyme [E_0] exists in the reaction medium as free enzyme [E], and bound enzyme [ES] and [EI]. In presence of inhibitor the Michaelis-Menten constant K_m of the normal reaction is increased to a new K_m ;[$\left(1+\frac{[I]}{K_i}\right)K_m$] , where the dissociation constant $K_i = \frac{[E][I]}{[EI]}$

The Michaelis-Menten equation for competitive inhibition becomes

$$V_0 = \frac{V_{max}[S]}{\left(1+\frac{[I]}{K_i}\right)K_m + [S]} \dots\dots\dots\dots\dots\dots (1)$$

Consequently, the double reciprocal form (Lineweaver-Burk) of the equation is modified as

$$\frac{1}{V_0} = \frac{\left(1+\frac{[I]}{K_i}\right)K_m}{V_{max}[S]} + \frac{1}{V_{max}} \dots\dots\dots\dots\dots\dots (2)$$

In presence of competitive inhibitor the slope of the double-reciprocal plot (fig-1) is increased to $\frac{\left(1+\frac{[I]}{K_i}\right)K_m}{V_{max}}$ but intercept with Y-axis remains same at $\frac{1}{V_{max}}$ while the intercept with X-axis at $-\frac{1}{\left(1+\frac{[I]}{K_i}\right)K_m}$ will differ according to the concentration of competitive inhibitors.

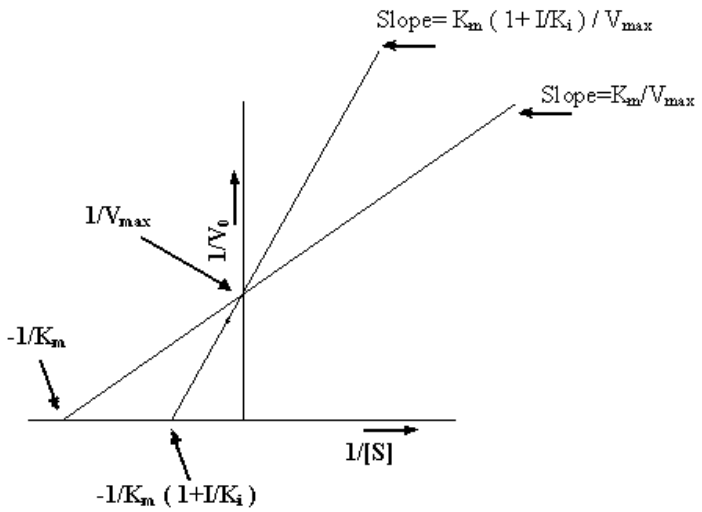


Fig-1: Lineweaver-Burk plot of uninhibited and competitive inhibited enzymes.

However, competitive inhibitors do not affect the turnover number (active site catalysis per unit time) or the efficiency of the enzyme because once the enzyme is free, it behaves normally.

Some examples of competitive inhibitors are sulfanilamides, a simplest form of sulfa drug. It is an antibiotic useful in the treatment of some kidney infection. As a structural analog of p-aminobenzoic acid (PABA) (fig-2), sulfanilamide inhibits bacterial growth. PABA is a structural part of folic acid. Thus sulfa drug is highly toxic to bacteria that must synthesize their own folic acid. Since humans require folate from dietary source, the sulfanilamide is not harmful at the dose that kills bacteria[4,5]. Methotrexate is another competitive inhibitor for dihydrofolate reductase. This drug closely resembles folic acid and it is used as anticancer antimetabolite chemotherapy particularly for paediatric leukemia. It hinders the availability of tetrahydrofolate which is important for anabolic pathways-particularly synthesis of purine nucleotides for DNA replication[5].

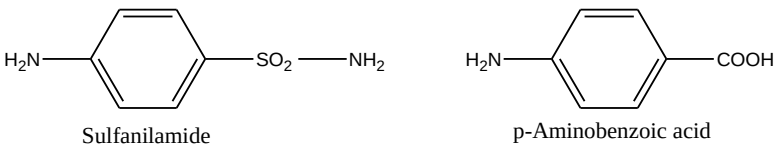
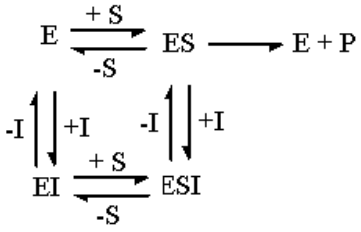


Fig.-2. *p*-Aminobenzoic acid substrate and sulfanilamide as a competitive inhibitor during the bacterial folate synthesis.

b) Non-competitive Inhibition

The non-competitive inhibitor does not have structural similarity to the substrate but it binds both the free enzyme and the enzyme-substrate complex. The simplified equation of steady-state kinetics of non-competitive inhibition is:



The presence of a substrate has no influence on the ability of a non-competitive inhibitor to bind an enzyme and vice versa. Its binding alters the conformation of the enzyme and reduces its catalytic activity due to changes in the nature of the catalytic groups at the active site. EI and ESI complexes are non-productive. On increasing the substrate to a saturating concentration does not reverse the inhibition leading to unaltered K_m but reduced V_{max}. Reversal of the inhibition requires a special treatment, eg. dialysis or pH adjustment.

Considering the steady-state kinetics of the non-competitive inhibition the dissociation constant or inhibitor constant (K_i) for the reactions, E+I↔EI and ES+I↔ESI is as follows:

$$K_i = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (3)$$

The total enzyme [E_0] exists in reaction mixture in four forms and presented as sum of concentrations of those four forms as follows:

$$[E_0] = [E] + [EI] + [ES] + [ESI] \dots\dots\dots\dots\dots (4)$$

Substituting equation (1) in equation (2), [E] can be expressed as:

$$[E] = \frac{[E_0]}{\left(1+\frac{[I]}{K_i}\right)} - [ES] \dots\dots\dots\dots\dots (5)$$

Considering the above equation, the Michaelis-Menten equation becomes:

$$V_0 = \frac{V_{max}}{\left(1+\frac{[I]}{K_i}\right)} \cdot \frac{[S]}{([S]+K_m)} \dots\dots\dots\dots\dots\dots (6)$$

The double reciprocal (Lineweaver-Burk) equation for simple linear non-competitive inhibitor is given as:

$$\frac{1}{V_0} = \frac{K_m}{V_{max}}\left(1+\frac{[I]}{K_i}\right) \cdot \frac{1}{[S]} + \frac{1}{V_{max}}\left(1+\frac{[I]}{K_i}\right) \dots\dots\dots\dots\dots (7)$$

The effect of non-competitive inhibitor on the double reciprocal plot of substrate-reaction rate is shown in fig-3. This type of inhibitor lowers the V_{max} but does not affect the K_m values.

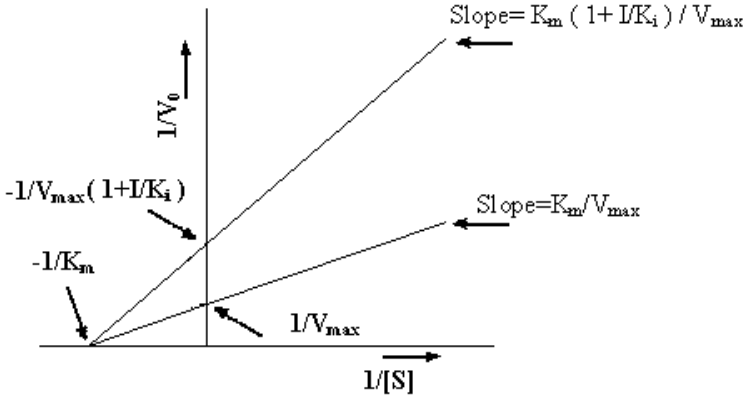


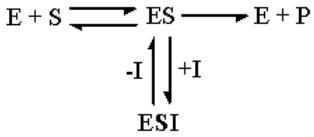
Fig-3: Lineweaver-Burk plot of uninhibited and non-competitive inhibited enzymes.

Examples of non-competitive inhibitors are mostly poisons. Cyanide and azide inhibit enzymes with iron or copper as a component of the active site or the prosthetic group, e.g. cytochrome c oxidase. Cyanide binds to the Fe^{3+} in the heme of the cytochrome aa_3 component of cytochrome c oxidase and prevents electron transport to O_2. Mitochondrial respiration and energy production cease, and cell death rapidly occurs. The central nervous system in the primary target for cyanide toxicity[6,7]. Acute inhalation of high concentrations of cyanide (e.g., smoke inhalation during a fire and automobile exhaust) provokes a brief central nervous system stimulation rapidly followed by convulsion, coma and death. Acute exposure to lower amounts can cause lightheadedness, breathlessness, dizziness, numbness and headaches. Cyanide is present in the air as hydrogen cyanide (HCN), in soil and water as cyanide salts (e.g., NaCN), and in foods as cyanoglycosides.

c) Uncompetitive Inhibition

The uncompetitive inhibitor has no structural similarity to the substrate. It may bind the free enzyme or enzyme-substrate complex that exposes the inhibitor binding site. Its binding, although away from the active site, causes structural distortion of the active and allosteric sites of the complexed enzyme that inactivates the catalysis. This leads to a decrease in both K_m and V_{max}. This inhibition cannot be reversed by increasing the substrate towards a saturating concentration and reversal requires special treatment like dialysis. This type of inhibition is rare and is encountered in multi-substrate enzymes, where the inhibitor competes with one

substrate (S_2) to which it has some structural similarity and is uncompetitive for the other (S_1). The reaction without the inhibitor would be- $E + S_1 \leftrightarrow ES_1 + S_2 \leftrightarrow ES_1S_2 \leftrightarrow E+Ps$ and with uncompetitive inhibitor becomes; $E+S_1 \leftrightarrow ES_1 + I \leftrightarrow ES_1I$ (prevents S_2 binding and produces no product). The steady-state kinetics of the uncompetitive inhibition reaction is as follows:



Considering steady-state kinetics of the uncompetitive inhibition, the dissociation constant, $K_i = \frac{[ES][I]}{[ESI]}$. Here the total enzyme [E_0] exists in the reaction mixture in three forms free enzyme [E], and bound enzymes [ES] and [ESI]. Kinetically, uncompetitive inhibition modifies the Michaelis-Menten equation as:

$$V_0 = \frac{V_{max}[s]}{K_i + \left(1 + \frac{[I]}{K_i}\right)[S]} \dots\dots\dots\dots\dots\dots (8)$$

and the double reciprocal equation becomes :

$$\frac{1}{V_0} = \frac{1 + \frac{[I]}{K_i}}{V_{max}} + \frac{K_m}{V_{max}}\frac{1}{[S]} \dots\dots\dots\dots\dots\dots (9)$$

The double-reciprocal plot of substrate reaction rate in presence of uncompetitive inhibitor is given in Fig.-4.

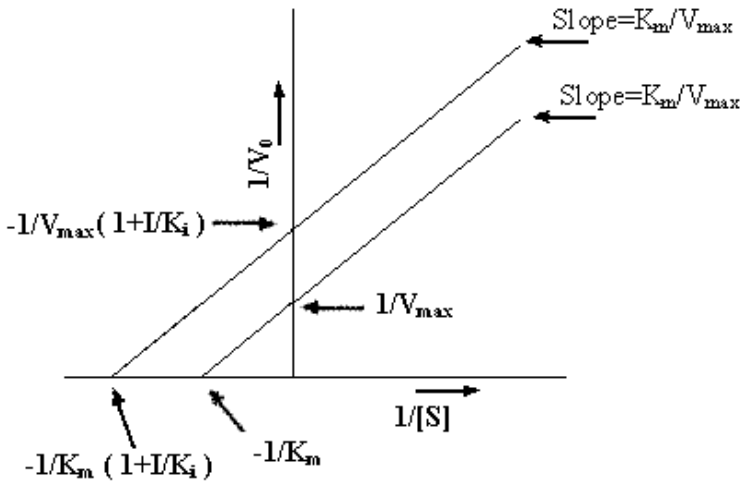


Fig.-4: Lineweaver-Burk plot of uninhibited and uncompetitive inhibited enzymes.

Uncompetitive reversible inhibition is rare but may occur in multimeric enzymes. Some examples of uncompetitive reversible inhibitors are the inhibition of the key regulatory heme synthetic enzyme, δ-aminolevulinate synthase and dehydratase and heme synthetase by heavy metal ion, e.g., lead. Heavy metals like lead from mercaptides with -SH at the active site of the enzyme (2R-SH + Pb → R-S-Pb-S-R + 2H). Oxidising agents like ferricynaide also oxidises -SH into a disulphide linkage (2R-SH → R-S-S-R). The ability of lithium to exert profound and selective psychopharmacological effects has been the focus of considerable research effort. There is increasing evidence that lithium exerts its therapeutic action by interfering with polyphosphoinositide metabolism in brain and prevention of inositol recycling by an uncompetitive inhibition of inositol monophosphatase[8]. Few pesticides are uncompetitive inhibitors, the best known example being the herbicide N-phosphomethylglycine, commonly known as glyphosate or round up, an uncompetitive inhibitor of 3-phosphoshikimate 1-carboxyvinyl transferase[9].

3. Irreversible Inhibition

The irreversible inhibitors have no structural relationship to the substrate and bind covalently with the active site of the enzyme or destroy an essential functional group of active site. So, irreversible inhibitors are used to identify functional groups of the enzyme active sites at the location they are bound. Subsets of irreversible inhibitors, called suicide irreversible inhibitors are relatively inactive compounds. They get activated upon binding with the active site of a specific enzyme. However, it does not release any product because of its irreversible binding at the enzyme active site. The enzyme in effect commits suicide by activating the inhibitor. Due to this very nature, suicide irreversible inhibitors are also called mechanism based activators or transition state analog inhibitors. The transition state analogs are extremely potent and specific inhibitors because they have higher affinity and stronger binding to the active site of the target enzyme than the natural substrate or products. However, exact design of drugs that precisely mimic the transition state is a challenge because of unstable structure of the transition state in the enzyme-catalysed reaction.

Kinetically, the irreversible inhibitors decrease the concentration of active enzyme and in turn decrease the maximum possible concentration of enzyme-substrate complex. It reduces the

initial concentration of enzyme [E_0] until all the enzymes are consumed to [E_0]-[I_0] level. Once the process between enzyme and the inhibitor gets completed and no free inhibitor is present in the mixture, further addition of the substrate follows the Michaelis-Menten equation. In absence of the inhibitor, the rate of formation of product from the excess substrate is $V_{max} = k_{cat}[E_0]$. While in presence of the inhibitor, the rate of formation of the product from the excess substrate is $V^i_{max} = k_{cat}\{[E_0] - [I_0]\}$. Here k_{cat} is the turnover number which is defined as the maximum number of molecules of substrate that an enzyme can convert to product per catalytic site per unit time. Combining the above two equations the following expression is obtained as:

$$\frac{V^i_{max}}{V_{max}} = \frac{K_{cat}\{[E_0] - [I_0]\}}{K_{cat}[E_0]} \dots\dots\dots\dots\dots\dots (10)$$

Which, in turn reduces to

$$V^i_{max} = V_{max}\left(1 - \frac{[I_0]}{[E_0]}\right) \dots\dots\dots\dots\dots\dots (11)$$

Thus V_{max} is reduced to V^i_{max} in presence of irreversible inhibition . This is similar to the reversible non-competitive inhibition, but in irreversible inhibition the relationship between V_{max} and V^i_{max} does not involve K_i.

Some clinical examples of suicide inhibitor is N,N-dimethylpropergylamine . This compound inhibits enzyme monoamine oxidase (MAO). MAO is responsible for breaking down neurotransmitter such as dopamine and serotonin and thus decreasing their concentration in the brain. Diseases such as Parkinson's disease and depression occur because of decrease level of dopamine and serotonin[10].

4. Conclusion

Enzyme inhibition is significant biological process to characterize the enzyme reaction, extraction of catalysis parameters in bio-industry and bioengineering. Conceptual modes of inhibition define the interactions of substrate-enzyme or inhibitor-enzyme or both substrate-enzyme-inhibitor in the moiety of active site. In recent years, application of enzymes and enzyme inhibition science have risen in healthcare, pharmaceutical, bio industries, environment and biochemical enzyme chip industries. New information is available in biochemistry about enzyme inhibitors and classes of enzyme inhibitory products with broad potential in therapeutic application in large markets. A better understanding of the emerging enzyme inhibitors on enzyme mechanism is the main key. Immobilized enzyme technology

has given a new way to economic tools in drug discovery and biosensor industry. The study of catalytic mechanism and regulation of catalytic activity is vital to understand the kinetics of enzyme inhibition which is fundamental to the modern design of pharmaceuticals in industry. Every year new enzyme inhibitors are discovered but success still needs to minimize challenges.

References:

1. A. Lehninger, D Nelson, M Cox. *Principles of Biochemistry*, Worth publishers, New York, (1993).
2. S. C. Rastogi, *Biochemistry*, Tata Mc. Graw- Hill, (2006).
3. N. K. Prasad, *Enzyme Technology*, PHI Learning Pvt. Ltd., (2011).
4. J. L. York., *Text book biochemistry with clinical corelations*, New York, (1997).
5. G. Bjelakovic et.al., Facta Universitatis, *Series Medicine and Biology*, **9(3),** 201-206, (2002).
6. L. L. Pearce, E. L. Bominaar, B. C. Hill & J. Peterson, *Journal of Biological Chemistry*, **278.,** 52139-52145, (2003).
7. H. B. Leavesley, L. Li, K.Pravakaran, J. L. Borowitz & G. E. Ison, *Toxicological Sciences*, **101(1).,** 101-111, (2008).
8. S.R. Naharski, et.al , *Trends Pharmacol Science*, **12(8).,** 297-303,(1991).
9. R.Eisenthal and A.Cornish-Bowden, *The Journal of Biological Chemistry*, **273(10).,** 5500-5505,(1998).
10. M. B. H. Youdim and Y. S. Bakhle, *British Journal of Pharmacology*, **147.,** 287-296, (2006).