

KINETICS OF ENZYME REACTIONS

Structure

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12.1 INTRODUCTION

In the previous unit you have learnt about the kinetics of catalytic reactions wherein you have learnt about catalysis, its characteristics, adsorption isotherms besides the mechanism and kinetics of homogeneous and heterogeneous catalytic reactions. You know that catalysts alter (generally increase) the rate of a chemical reaction by providing an alternate mechanism of different (generally lower) activation energy. In this unit we are going to take up the enzyme catalysed reactions and their kinetics. You are aware that enzymes are biological catalysts that speed up chemical reactions in living organisms.

We will start the unit by discussing about enzymes, their characteristics, the related terminology, and mechanism of their action. This will be followed by a discussion on the mathematical description of the mechanism of enzyme catalysed reactions. In this context we will take up the Michaelis–Menten mechanism and derive and analyse the corresponding expression for the rate of reaction. Thereafter, we will take up a very important aspect of enzyme catalysis i.e., enzyme inhibition. Herein, we will take up different types of enzyme inhibition mechanisms and learn about their kinetic characteristics. These will be compared with those of uninhibited reactions.

In the next unit you will learn about Debye-Huckel theory.

Expected Learning Outcomes

After studying this unit, you should be able to:

- ❖ define enzymes and outline their characteristics;
- ❖ give the Michaelis–Menten mechanism of enzyme catalysed reactions;
- ❖ derive an expression for the rate of enzymatic reaction based on Michaelis–Menten mechanism;
- ❖ explain the significance of the terms like turnover number and Michaelis constant;
- ❖ transform Michaelis–Menten rate law into Lineweaver–Burk equation, and explain the significance of Lineweaver–Burk plot;
- ❖ discuss ways of graphical analysis of Michaelis–Menten equation;
- ❖ define enzyme inhibition and state its different types;
- ❖ explain different types of enzyme inhibition mechanisms,
- ❖ derive rate equations for different mechanisms of enzyme inhibition and analyse the same; and
- ❖ state and compare characteristic kinetic features of different types of enzyme inhibitions.

12.2 ENZYMES AND THEIR CHARACTERISTICS

You have learnt in your earlier classes that enzymes are biological catalysts that speed up chemical reactions in living organisms. Virtually all the reactions occurring within a cell require the action of an enzyme as most of these reactions will not occur at an appreciable rate under physiological conditions of the cell. The term enzyme was introduced by W. Kühne in 1878 to refer to certain substances in yeast (Greek: en, in zyme, yeast) that made it cause fermentation. The enzymes are mostly proteins (certain ribonucleic acid molecules also act as enzymes) and are found in all forms of life, from bacteria to plants and animals. The fact that enzymes are proteins was established by American biochemist James Sumner in 1926 who crystallized jack bean *urease*—the enzyme that catalyses the cleavage of urea to ammonia and carbon dioxide and showed it to be a protein. The enzymes act by providing an alternate path of lower activation energy for a reaction to occur at much higher rates than those in their absence. Like catalysts, they also increase the rate of reactions without themselves being consumed or permanently altered by the reaction and without altering the chemical equilibrium between reactants and products.

Since most of the enzymes are proteins, these work at a specific temperature or within a narrow range of temperatures. At higher temperatures, the enzyme may get denatured and hence lose activity. Similarly, they have maximum activity at an optimum pH; the enzyme activity decreases at pH higher or lower than this. Enzyme catalysed reactions occur under relatively mild conditions:

temperatures below 100°C, atmospheric pressure, and nearly neutral pH whereas chemical catalysts often require harsher conditions of temperatures, pressures and pH. Further, like catalysts, the enzymes are required in very small amounts to affect large quantities of reactants. However, if the conditions are changed and become unfavourable, they can be poisoned. Let us take up important characteristics of enzymes.

Characteristics of enzymes

The important characteristics of enzymes are:

- a) Catalytic power
- b) Specificity and
- c) Regulation

You would have learnt about these in your earlier classes. We will briefly recall them here.

a) Catalytic power

The enzymes have a very high catalytic power; these may increase the rate of a reaction by about 10^6 to 10^{12} times that in their absence. That is, the rates of enzyme catalysed reactions may be several orders of magnitude greater than those of the corresponding chemically catalysed reactions. For example, a single molecule of the enzyme, *catalase* can catalyse the decomposition of about 10 million molecules of hydrogen peroxide in one second. The enzymes achieve these high rates by altering the thermodynamics of a reaction in such a way that the reactants and products reach equilibrium much faster than in their absence, without altering their equilibrium concentrations. The catalytic activity of the enzymes depends on their native three-dimensional structure; any slight variation in it may result in significant changes in their activity.

b) Specificity

The nature has developed specific enzymes to facilitate most of the biological reactions required for the survival of the organism. Enzymes demonstrate high reaction specificity. For example, the enzyme *urease* catalyses only the hydrolysis of urea, and no other reaction. This is an example of **absolute specificity**. The enzymes are so specific that if two different types of products can be obtained from a given substrate then we will have two different enzymes, each giving a specific product. However, all enzymes are not restricted to a particular reaction; some of them catalyse a class of reactions. They have specificity towards a kind of bond or linkage in the substrate rather than the substrate itself. For example, the hydrolysis of many phosphate esters, independent of the molecular groups attached to the ester linkage, are catalysed by an enzyme called *phosphatase*. Such enzymes are said to represent **linkage specificity**. Some enzymes catalyse reactions of only one stereoisomer of a compound; they have **stereochemical specificity**. For example, proteases only hydrolyse the derivatives of L-amino acids and not the corresponding D-amino acids. Some enzymes act only on molecules having specific functional groups and are said to have **group specificity**.

The specificity of an enzyme is closely related to its three-dimensional structure i.e., the tertiary structure.

c) Regulation

In living systems, hundreds of different enzyme catalysed reactions occur simultaneously. To coordinate different metabolic processes in the living cell it is important to regulate their activity. Regulatory enzymes exhibit increased or decreased catalytic activity in response to certain signals. The enzymes can be regulated by controlling their concentration or the availability of substrate. The catalytic activities of many enzymes vary in response to the concentrations of substances other than their substrates and can therefore be used for regulation of their activity.

Having recalled the important characteristics of enzymes let us also recall the mechanism of their action.

Mechanism of enzyme action

The mechanism of action of enzymes involves the binding of the reactants (called **substrates**) to a part of enzyme surface called **active site**. The shape of the active site and the substrate on which the enzyme acts are complementary and thus explain the specificity. This idea was suggested by German Chemist Emil Fischer in 1894 in terms of a 'lock and key' hypothesis; the enzyme being the lock and the substrate, the key. Like a lock has a specific key and vice versa, an enzyme mostly works best on a specific substrate as discussed above.

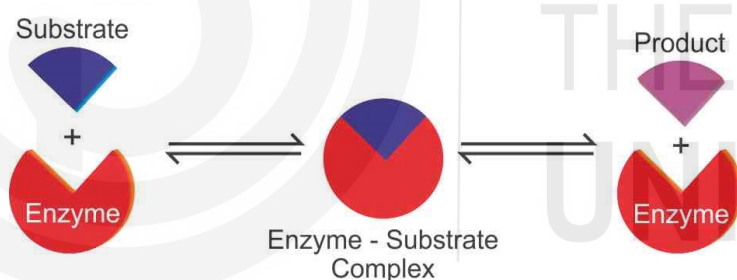


Fig. 12.1: Schematic representation of Emil Fischer's Lock and Key hypothesis of enzyme action.

This hypothesis considers the enzyme to have a rigid structure. However, now we know that the protein structure is not rigid but dynamic. To accommodate the dynamic nature of protein molecules Koshland proposed an alternative hypothesis called '**induced-fit**' hypothesis. According to this hypothesis, the binding of the substrate to the enzyme causes alteration in the geometry of the enzyme such that the appropriate groups in the substrate and in the enzyme are in the correct orientation with respect to each other. This hypothesis can be understood in terms of *hand in glove analogy*. Like when we place the hand in the glove, it acquires the shape of the hand. Having recalled the general characteristics of enzymes let us take up Michaelis-Menten mechanism that describes the general principles of enzyme kinetics.

However, before that answer the following simple questions to assess your understanding.

SAQ 1

What are enzymes? List three important characteristics of enzymes.

SAQ 2

In what way is Koshland's hypothesis on mechanism of enzyme action different from that of Emil Fischer's hypothesis?

12.3 THE MICHAELIS-MENTEN MECHANISM

Let us now consider a simple enzymatic reaction, involving the conversion of a substrate, S to a product, P catalyzed by an enzyme, E. Although most enzymatic reactions have two or more substrates, the general principles of enzyme kinetics can be described by assuming the simple case of one substrate and one product. In this context we will take up Michaelis-Menten mechanism-a model that describes the kinetics of such simple enzyme reactions. It was proposed by Leonor Michaelis and Maud Menten in 1913 and gives a simple mathematical treatment of such enzyme reactions. It provides the relationship between the rate of an enzyme catalysed reaction and the concentrations of the enzyme and the substrate. The treatment is based on the following assumptions:

- The enzymes bind to their substrate in a reversible manner to form an enzyme-substrate complex, ES which then gives the product in the second step. The rate of the reaction is directly proportional to the amount of enzyme-substrate complex so formed.
- The concentration of enzyme [E], is very small as compared to that of the substrate, [S] such that the formation of ES complex does not significantly decrease substrate concentration. For example, if the concentration of substrate is 10^{-3} M and that of the enzyme is 10^{-9} M, then even when all the enzyme is complexed with the substrate, the concentration of free substrate does not change significantly.
- The concentration of the product [P] is effectively zero. The initial concentration of the product is naturally zero and since we measure the initial rate of the reaction, the amount of P formed in the time of measurement of the 'initial-rate' is too small to give rise to a significant reverse reaction
- The reverse reaction of enzyme-substrate complex formation is faster than the formation of product from the ES complex.

Thus, the reaction takes place in two distinct steps: the formation of the enzyme-substrate complex, and the actual chemical reaction accompanied by the dissociation of the product from the enzyme. The mechanism can be represented as under



where P represents the product, k_1 and k_2 are rate constants for the forward reactions, and k_{-1} is rate constant for the reverse reaction. The mathematical expression describing the kinetics of this mechanism can be obtained by applying the steady-state approximation to the complex [ES]. At steady state, the rate of the reaction giving the product (k_2 [ES]) plus that for decomposition of enzyme-substrate complex (k_{-1} [ES]) becomes equal to the rate of the formation of the enzyme-substrate complex, (k_1 [E][S]). That is, the ES complex is formed at the same rate that it decomposes, so that the concentration of ES stays steady (constant). We can write

$$k_1[E][S] - k_{-1}[ES] - k_2[ES] = 0 \quad \dots (12.2)$$

It is difficult to ascertain the concentrations of free enzyme and the enzyme-substrate complex during the reaction. Therefore, we use the terms that can be measured, such as the rate constant, total enzyme concentration and substrate concentration for arriving at the rate expression.

Now, since the total concentration of enzyme (free + bound) is equal to its initial concentration, $[E]_0$, we can write

$$[E]_0 = [E] + [ES] \quad \dots (12.3)$$

This gives,

$$[E] = [E]_0 - [ES] \quad \dots (12.4)$$

Substituting in Eq. (12. 2) we get

$$k_1([E]_0 - [ES])[S] - (k_{-1} + k_2)[ES] = 0 \quad \dots (12.5)$$

Simplifying, we get

$$[ES] = \frac{k_1[E]_0[S]}{k_{-1} + k_2 + k_1[S]} \quad \dots (12.6)$$

This equation describes the steady-state concentration of complex ES using terms that can be measured in an experiment. As per the assumptions given above, the rate of product formation is

$$\text{Rate, } R = k_2 [ES] \quad \dots (12.7)$$

Substituting for [ES] from Eq. (12.6), we get the reaction rate in terms of initial enzyme concentration and the concentration of the substrate

$$R = \frac{k_1 k_2 [E]_0 [S]}{k_{-1} + k_2 + k_1 [S]} \quad \dots (12.8)$$

We can simplify the expression by dividing the numerator and the denominator of the fraction by k_1 , to get

$$R = \frac{k_2 [E]_0 [S]}{\left(\frac{k_{-1} + k_2}{k_1} \right) + [S]} \quad \dots (12.9)$$

The ratio of the constants in the denominator is called the **Michaelis constant** and is represented as K_M .

$$K_M = \left(\frac{k_{-1} + k_2}{k_1} \right) \quad \dots (12.10)$$

When $k_{-1} \gg k_2$ i.e., the rate of dissociation of ES complex to enzyme and substrate is more than that of the formation of the product from ES. In such a case, Michaelis constant equals the dissociation constant of the enzyme-substrate complex. Thus, a low value of K_M corresponds to a strong enzyme-substrate complex. In other words, the value of Michaelis constant gives an idea of strength of binding and saturation of enzyme and substrate. For given enzyme, its value depends on the substrate and the experimental conditions like pH, temperature, solvent, ionic strength etc.

We can express the reaction rate or Michaelis Menten equation in terms of the Michaelis constant, as

$$R = \frac{k_2 [E]_0 [S]}{K_M + [S]} \quad \dots (12.11)$$

When the concentration of the substrate is small i.e., $[S] \ll K_M$ we can write

$$R \sim \frac{k_2}{K_M} [E]_0 [S] \quad \dots (12.12)$$

That is, at low substrate concentrations, the reaction rate is first order with respect to the substrate concentration. A plot of rate versus substrate concentration will be linear at lower substrate concentrations. However, for high substrate concentration, i.e., $K_M \ll [S]$ we can write,

$$R \sim k_2 [E]_0 \quad \dots (12.13)$$

That is, at high substrate concentrations, the reaction is zero order with respect to the substrate i.e., the rate does not depend on the substrate concentration. The variation of the initial rate of reaction as a function of substrate concentration is schematically shown in Fig 12.2.

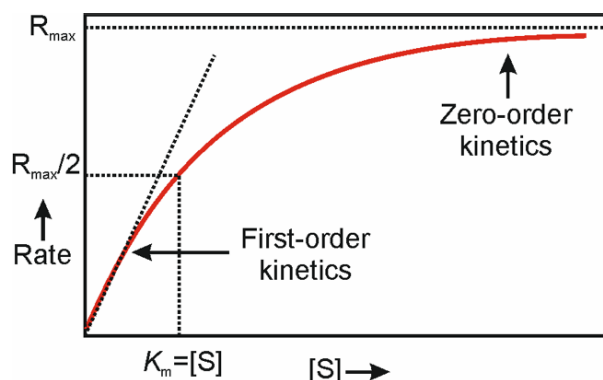


Fig. 12.2: A plot of reaction rate versus substrate concentration for an enzyme reaction following Michaelis–Menten kinetics.

The zero-order dependence is observed at high substrate concentration because at this stage the enzyme is saturated i.e., the concentration of substrate is sufficient to complex with all the enzyme i.e., all the enzyme is present as ES. As fast as ES complex breaks down to release E and P, or E

and S, the available excess substrate converts the freed enzyme back to enzyme-substrate complex. Therefore, any further increase in concentration of the substrate [S] is not reflected in the effects on the reaction rate.

You will recall that this behaviour is like that of heterogeneous surface catalysis. In both the cases the reactant binds to a site, an active site on the enzyme and free valence on the surface of a solid. In both the cases we observe a transition from first order to zero-order kinetics as the reaction proceeds. The Eq. (12.11) can be represented in another form, using the maximum rate, R_{max} which is defined as

$$R_{max} = k_2 [E]_0 \quad \dots (12.14)$$

This suggests that the rate of product formation will plateau at a certain maximum value equal to the product of initial enzyme concentration and k_2 , the rate constant for product formation. A simplified version of Michaelis-Menten equations can be written by using both K_M and R_{max} as follows

$$R = \frac{R_{max} [S]}{K_M + [S]} \quad \dots (12.15)$$

Now, when $K_M = [S]$ then

$$R = R_{max} / 2. \quad \dots (12.16)$$

This expression provides an alternative meaning to the Michaelis constant i.e., when $K_M = [S]$ the initial rate is half of the maximum rate, shown graphically in Fig. 12.3. Thus, K_M (in mol dm^{-3}) is equal to the concentration of the substrate at which the initial rate of the enzyme catalysed reaction is half of its maximum value.

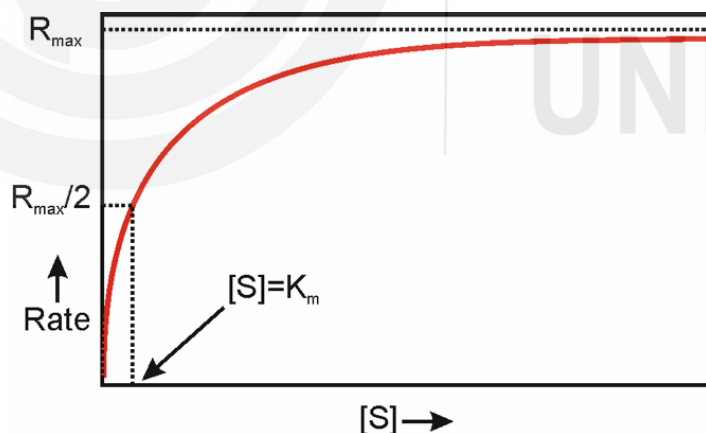


Fig. 12.3: Schematic representation of the dependence of the initial reaction rate of a simple Michaelis-Menten scheme on the substrate concentration.

It is also useful to define another constant called **catalytic constant** of an enzyme as follows:

$$k_{cat} = \frac{R_{max}}{[E]_0} \quad \dots (12.17)$$

The catalytic constant measures how quickly a given enzyme can catalyse a specific reaction; it's a very useful way of describing the effectiveness of an

enzyme. This quantity is also known as the **turnover number** of an enzyme. It represents the number of reaction processes (turnovers) that the active site of the enzyme catalyses per unit time. In other words, the turnover number of an enzyme is the number of substrate molecules or the moles of the substrate that is converted to product per second per mole of enzyme (or per mole of active site for a multi-subunit enzyme) when the enzyme is fully saturated with the substrate. Under physiological conditions, for most enzymes, the turnover number ranges from 1 to 10^6 s^{-1} . The unit of catalytic constant as s^{-1} can be rationalised by the fact that it is a ratio of rate (mol s^{-1}) and initial enzyme concentration (mol).

We have seen above that Michaelis–Menten equation (Eq. (12.11)) has two limiting scenarios. That is at low substrate concentrations the rate increases linearly (first order) with substrate concentration and at high substrate concentrations it becomes independent (zero order) of the substrate concentration. These two effects can be demonstrated in a comprehensive way by integration of the rate expression.

We can write the Michaelis–Menten equation in differential form as

$$-\frac{d[S]}{dt} = \frac{k_2 [E]_0 [S]}{K_M + [S]} \quad \dots (12.18)$$

Dividing both sides by $\frac{[S]}{K_M + [S]}$ we get,

$$\frac{-\frac{d[S]}{dt}}{\frac{[S]}{K_M + [S]}} = k_2 [E]_0 \quad \dots (12.19)$$

This can be simplified to

$$-\frac{d[S]}{dt} \cdot \frac{K_M + [S]}{[S]} = k_2 [E]_0 \quad \dots (12.20)$$

Rearranging we get

$$-d[S] \left(\frac{K_M}{[S]} + 1 \right) = k_2 [E]_0 dt \quad \dots (12.21)$$

Simplifying again, we get

$$-K_M \cdot \frac{d[S]}{[S]} - d[S] = k_2 [E]_0 dt \quad \dots (12.22)$$

Integrating Eq. (12.22) in limits of $[S]_0$ at $t = 0$ and $[S]$ at time t , we get

$$K_M \ln \frac{[S]_0}{[S]} + ([S]_0 - [S]) = k_2 [E]_0 t \quad \dots (12.23)$$

It is interesting to note that the first term on the left-hand side of the equation shows the first-order dependence of the rate, whereas the second term shows the zero-order dependence.

12.3.1 Analysing Michaelis–Menten Equation

To put Eq. (12.15) to use we need to know the values of R_{max} and, K_M . These can be obtained by measuring the initial rate using several different concentrations of S. However, it is not easy, to draw a hyperbola using a set of experimental points, especially when the measurements do not extend to the whole range. This may be due to the insolubility of the substrate or it being expensive. Therefore, in the absence of an accurate extrapolated value of R_{max} it becomes difficult to estimate K_M . This problem can be solved by converting the equation into a suitable linear form. With linearisation we can extrapolate the experimental data to a reasonable extent to get the kinetic constants.

Several methods have been developed for analysing data for the rates of enzyme catalysed reactions. In one of these methods, the Eq. (12.11) is written in the reciprocal form as follows

$$\frac{1}{R} = \frac{K_M + [S]}{k_2 [E]_0 [S]} = \frac{K_M}{k_2 [E]_0 [S]} + \frac{[S]}{k_2 [E]_0 [S]} \quad \dots (12.24)$$

This can be simplified to

$$\frac{1}{R} = \frac{K_M}{k_2 [E]_0 [S]} + \frac{1}{k_2 [E]_0} \quad \dots (12.25)$$

This implies that a plot of $\frac{1}{R}$ versus $1/[S]$, the reciprocal of the substrate

concentration, will give a straight line. The slope of the line will be $\frac{K_M}{k_2 [E]_0}$ and

the intercept will be $\frac{1}{k_2 [E]_0}$. Such a plot, is known as a **Lineweaver-Burk plot**

and is schematically given in Fig.12.4. Since the plot is between reciprocals of the initial rate and the substrate concentration, it is also called as a **double reciprocal plot** and is quite useful. The intercept provides the value of R_{max} which can then be used to get the value of K_M from the slope.

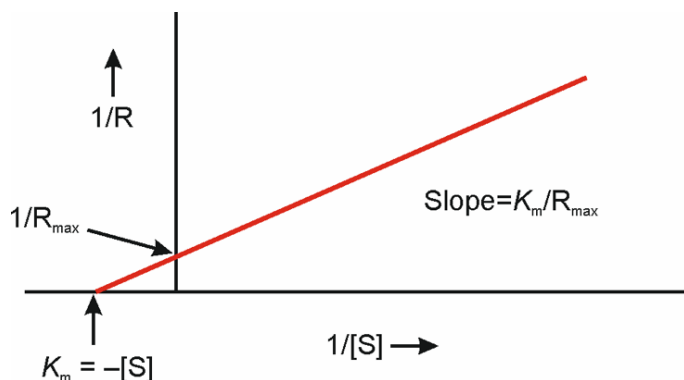


Fig. 12.4: A Lineweaver-Burk or double reciprocal plot for an enzyme-catalyzed reaction.

However, it has a drawback that since most of the data is for relatively high substrate concentrations, the extrapolation of the line to low $[S]$ may have an inherent error.

We can modify **Lineweaver-Burk expression** [Eq. (12.25)] by multiplying with [S], to get

$$\frac{[S]}{R} = \frac{[S]}{k_2 [E]_0} + \frac{K_M}{k_2 [E]_0} \quad \dots (12.26)$$

According to Eq. (12.26), a plot of $\frac{[S]}{R}$ versus [S] will be a straight line with a slope of $\frac{1}{k_2 [E]_0}$ which is equal to $\frac{1}{R_{\max}}$ and an intercept of $\frac{K_M}{k_2 [E]_0}$ which is $\frac{K_M}{R_{\max}}$. Such a plot is known as a **Hanes-Wolf plot** (single reciprocal plot) and is schematically given in Fig. (12.5).

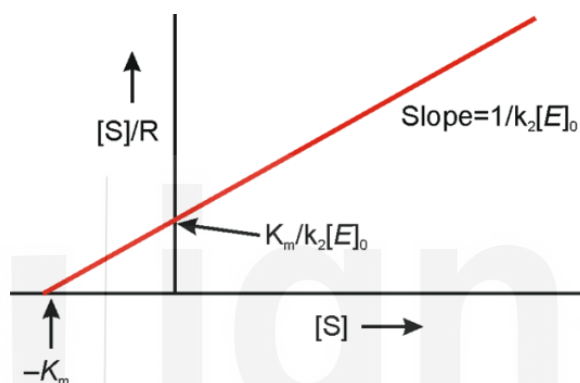


Fig. 12.5: A schematic Hans-Wolff plot.

The value of R_{\max} is obtained from the slope and the value of K_M can be obtained from the intercept by using the value of R_{\max} . You may note that it is opposite of Lineweaver-Burk plot where the intercept gives R_{\max} and K_M is obtained from slope by using R_{\max} .

A yet another way of analysing data for enzyme-catalyzed reactions can be obtained by rewriting Eq. (12.11) in the following form

$$R(K_M + [S]) = k_2 [E]_0 [S] \quad \dots (12.27)$$

Simplifying, we get

$$RK_M + R[S] = k_2 [E]_0 [S] \quad \dots (12.28)$$

Dividing both sides of the equation by substrate concentration, [S] we get

$$\frac{RK_M}{[S]} + R = k_2 [E]_0 \quad \dots (12.29)$$

Rearranging

$$R = -\frac{RK_M}{[S]} + k_2 [E]_0 \quad \dots (12.30)$$

According to this equation, a plot of R versus $R/[S]$ will be a straight line with a slope of $-K_M$ and an intercept of $k_2 [E]_0$ i.e., R_{\max} . Such a plot is known as an **Eadie-Hofstee plot**, and it is schematically given in Fig. 12.6. You may note that this is also a single reciprocal plot.

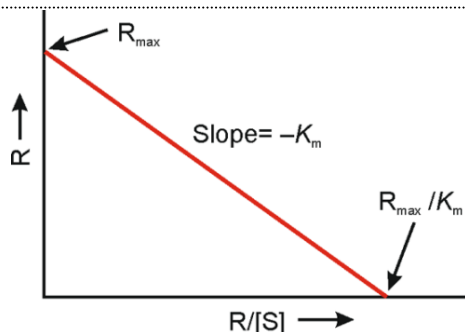


Fig. 12.6: A schematic Eadie-Hofstee plot.

Having learnt about the Michaelis–Menten mechanism describing the kinetic behaviour of enzyme catalysed reactions, let us now take up another important aspect of enzyme catalysed reactions viz., enzyme inhibition. However, before that answer the following simple questions to assess your understanding.

SAQ 3

Give an example each of single reciprocal and double reciprocal plots based on Michaelis–Menten equation.

SAQ 4

How do we determine the values of R_{\max} and K_M from Lineweaver-Burk plot?

12.4 MECHANISMS OF ENZYME INHIBITION

We have seen above that enzyme action is manifested in terms of the binding of the substrate molecule to the active site of the enzyme. For many enzymes, the active site is localised to a small region of the much larger molecule. If some substance other than the substrate becomes bound to the active site of the enzyme, part, or all the ability of the enzyme to function as a catalyst is lost. We say that the enzyme has been **inhibited**. For example, the catalytic activity of the enzyme *urease* that catalyses the conversion of urea to NH_3 and CO_2 is decreased by the presence of metal ions like, Ag^+ , Pt^{2+} , Hg^{2+} , and Pt^{2+} . That is, these metal ions act as enzyme inhibitors. Thus, we can define an **enzyme inhibitor (I)** as a compound that binds to an enzyme and interferes with its activity. The inhibitors can act either by preventing the formation of the ES complex or by blocking the chemical reaction that leads to the formation of product. Generally, the inhibitors are small molecules that bind reversibly to the enzyme they inhibit. Certain drugs act as inhibitors for the action of some enzymes. Cells contain many natural enzyme inhibitors that play important roles in regulating metabolism. Most biologically relevant enzyme inhibition is reversible in nature.

The reversible inhibitors are bound to enzymes by the same noncovalent forces that bind substrates and products to the enzyme. Though in some cases the inhibitors may bind irreversibly to the enzyme by covalent interactions. The action of inhibitors is to be differentiated from that of various non-specific agents (acid or alkali, urea, detergents, proteases etc.) which affect the enzyme activity by disrupting protein structure. The equilibrium between free enzyme (E), inhibitor (I) and the EI complex can be represented as



It is characterized by a constant called **inhibition constant**. It is important to understand the mechanisms and the kinetics of this important aspect of enzyme action for their practical importance in many ways. Some of these are:

- The phenomenon of reversible enzyme inhibition serves as a powerful tool for probing enzyme activity. The experiments involving a series of competitive inhibitors with systematically altered structures can provide information about the shape and chemical reactivity of the active site of an enzyme.
- Inhibition of enzymes by key metabolites serves as a means of metabolic fine control in the cell.
- The action of drugs, pesticides, undesirable toxic agents, etc. often depend on the inhibition of enzyme action.

There are three simple kinetic models for enzyme inhibition. These are:

- competitive inhibition
- uncompetitive inhibition, and
- non-competitive inhibition

These can be distinguished experimentally by their effects on the kinetic behaviour of the enzyme. We will take up the mathematical treatment of these inhibition mechanisms one by one. We begin with competitive inhibition.

12.4.1 Competitive Inhibition

You have learnt above that the enzyme action can be understood in terms of the active site hypothesis. If an inhibiting substance that can bind at the enzyme active site is present in the system there will be competition between the substrate, S, and the inhibitor, I, for the active site on the enzyme. The enzyme that is bound in a complex with the inhibitor, EI, is not available for binding with the substrate, so the effectiveness of the enzyme will be diminished. Similarly, an enzyme bound to the substrate is unavailable for the inhibitor. In other words, the free enzyme, E, combines either with S, to give the productive complex ES, or with I, to give EI, but not with both.

These inhibitors usually resemble the substrate i.e., they are substrate analogues so that they bind specifically to the active site, but they differ from

the substrate such that they do not react as the substrate does. Such inhibitors are called **competitive inhibitors**. These are the most encountered inhibitors in biochemistry. The competitive inhibition as discussed is called classical competitive inhibition and is shown pictorially in Fig. 12.7(a).

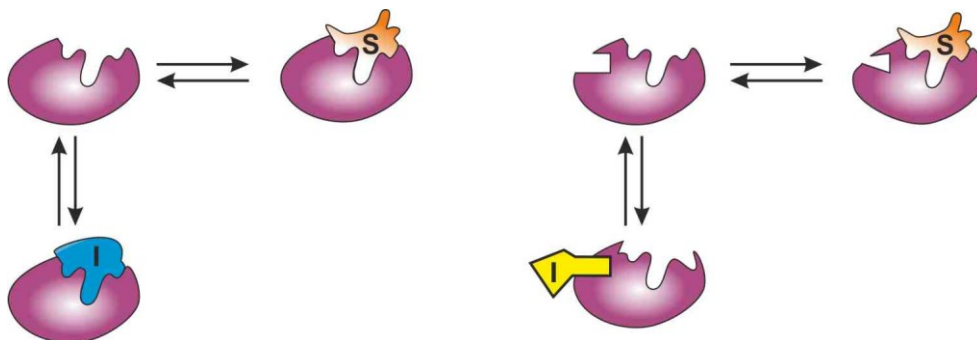
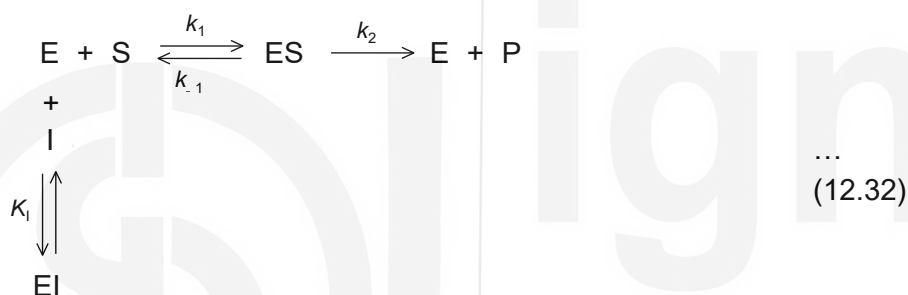


Fig. 12.7: A pictorial depiction of (a) classical competitive inhibition and (b) non-classical competitive inhibition.

The kinetic scheme for classical competitive inhibition can be represented as



In some cases, a compound quite dissimilar to the substrate may bind to the enzyme at a place other than the substrate binding site. This may alter the shape or charge distribution in the active site in a way that the substrate could no longer bind to it. Such an inhibition is called **competitive inhibition through conformational change** or **non-classical competitive inhibition**. It is pictorially represented in Fig. 12.7(b).

In competitive inhibition, S and I are mutually exclusive. At a given concentration of the substrate the enzyme is distributed between E and ES however, in presence of I some of the E is converted to EI. The balance between E and ES is disturbed which readjusts in favour of E. The net effect is that at the new equilibrium, the steady-state level of the productive complex ES, are reduced leading to a decrease in the rate. At very high concentrations of the inhibitor, all the enzyme converts into EI and the catalysis stops completely. Now since I can displace S, S can also displace I. Therefore, increasing the concentration of S, will release inhibitor from EI and make free E available to make more ES. At very high concentrations of S, all the enzyme will essentially be present as ES even in the presence of inhibitor. The maximum rate therefore is unaffected. Let us derive a mathematical expression describing competitive inhibition.

Mathematical expression for competitive inhibition

When both I and S are present in the system, the proportion of the enzyme forming ES complexes depends on the concentrations of substrate and

inhibitor, and their relative affinities for the enzyme. In this system, the concentration of “free” enzyme, $[E]$, is the total concentration, $[E]_0$ minus the amount bound in the ES and EI complexes. Therefore, a competitive inhibitor reduces the concentration of free enzyme available for substrate binding. To get the amount of the enzyme bound to the inhibitor we consider Eq. (12.31)



Writing the expression for the equilibrium constant and substituting for $[E]$ as $([E]_0 - [ES] - [EI])$, i.e., total enzyme concentration minus the amount bound to the substrate and to the inhibitor, gives

$$K = \frac{[EI]}{[E][I]} = \frac{[EI]}{([E]_0 - [ES] - [EI])[I]} \quad \dots (12.32)$$

If we let K_1 represent the equilibrium constant for *dissociation* of complex EI, then $K_1 = 1/K$, we can write

$$K_1 = \frac{1}{K} = \frac{([E]_0 - [ES] - [EI])[I]}{[EI]} \quad \dots (12.33)$$

Solving the expression for $[EI]$ gives

$$[EI] = \frac{[I]([E]_0 - [ES])}{K_1 + [I]} \quad \dots (12.34)$$

As in the case of Michaelis–Menten equation, the change in concentration of the complex ES with time equals the difference of the rate at which ES is formed and the rate at which it dissociates. Therefore, after a steady state is reached,

$$\frac{d[ES]}{dt} = k_1([E]_0 - [ES] - [EI])[S] - k_{-1}[ES] - k_2[ES] = 0 \quad \dots (12.35)$$

Where $[E]_0$ is the total concentration of the enzyme. Rearranging, we get

$$k_1([E]_0 - [ES] - [EI])[S] = (k_{-1} + k_2)[ES] \quad \dots (12.36)$$

Simplifying and substituting K_M for $\left(\frac{k_{-1} + k_2}{k_1}\right)$ we get

$$([E]_0 - [ES] - [EI])[S] = K_M [ES] \quad \dots (12.37)$$

Substituting for $[EI]$ from Eq. (12.34) and simplifying we get the following expression for $[ES]$.

$$[ES] = \frac{[E]_0 [S] K_1}{[S] K_1 + K_M K_1 + K_M [I]} \quad \dots (12.38)$$

Since the rate of formation of product is given by

$$R = k_2 [ES] \quad \dots (12.39)$$

We can write the rate expression as

$$R = \frac{k_2 [E]_0 [S] K_I}{[S] K_I + K_M K_I + K_M [I]} \quad \dots (12.40)$$

When [S] is large, the first term in the denominator is far greater than the other two, which could be neglected. The rate expression simplifies to $R = k_2 [E]_0$ i.e., the maximum rate; R_{max} .

Therefore, substituting for $k_2 [E]_0$ in Eq. (12.40) as R_{max} gives

$$R = \frac{R_{max} [S] K_I}{[S] K_I + K_M K_I + K_M [I]} \quad \dots (12.41)$$

Writing this equation in terms of $1/R$ and rearranging gives the double reciprocal or **Lineweaver-Burk** form

$$\frac{1}{R} = \frac{1}{R_{max}} \left(K_M + \frac{K_M [I]}{K_I} \right) \frac{1}{[S]} + \frac{1}{R_{max}} \quad \dots (12.42)$$

Which is usually written in the following form

$$\frac{1}{R} = \frac{K_M}{R_{max}} \left(1 + \frac{[I]}{K_I} \right) \frac{1}{[S]} + \frac{1}{R_{max}} \quad \dots (12.43)$$

This equation indicates that a graph of $1/R$ versus $1/[S]$ should be linear with a slope of $\frac{K_M}{R_{max}} \left(1 + \frac{[I]}{K_I} \right)$ and an intercept as $\frac{1}{R_{max}}$. A graphical representation of the same is given in Fig. 12. 8.

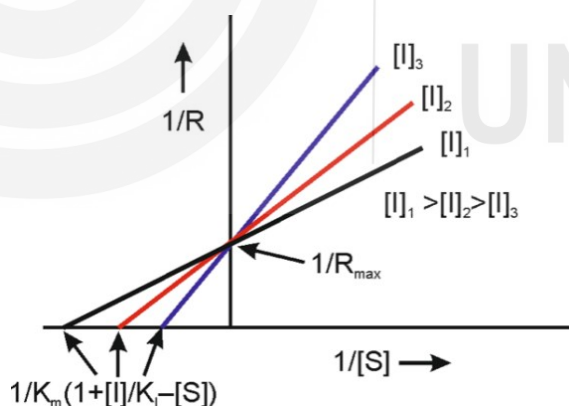


Fig. 12.8: A Lineweaver-Burk plot for the case of competitive enzyme inhibition at three different [I] concentrations of inhibitor. Note the common intercept characteristic of competitive inhibition.

The slope depends on the concentration of the inhibitor therefore, we will get a characteristic line for each concentration of the inhibitor used. On the other hand, the intercept is independent of the inhibitor concentration. Therefore, if on varying the inhibitor concentration we get a family of lines having a common intercept, it serves as a diagnostic test for competitive inhibition. In other words, it demonstrates that the inhibitor is functioning in a competitive manner

$$[ES] = \frac{[E]_0}{\left[\left(1 + \frac{[I]}{K_I} \right) + \frac{(k_{-1} + k_2)}{k_1[S]} \right]} \quad \dots (12.52)$$

Now rate, $R = k_2 [ES]$.

Substituting $[ES]$ from Eq. (12.52) we get

$$R = \frac{k_2 [E]_0}{\left(1 + \frac{[I]}{K_I} \right) + \frac{(k_{-1} + k_2)}{k_1[S]}} \quad \dots (12.53)$$

Multiplying the numerator and denominator by $[S]$ we get

$$R = \frac{k_2 [E]_0 [S]}{\left(1 + \frac{[I]}{K_I} \right) [S] + \frac{(k_{-1} + k_2)}{k_1}} \quad \dots (12.54)$$

Dividing the numerator and denominator by $\left(1 + \frac{[I]}{K_I} \right)$ we get

$$R = \frac{\frac{k_2 [E]_0 [S]}{\left(1 + \frac{[I]}{K_I} \right)}}{\left[S \right] + \frac{\left(\frac{k_{-1} + k_2}{k_1} \right)}{\left(1 + \frac{[I]}{K_I} \right)}} \quad \dots (12.55)$$

A close look at the Eq. (12.55) and comparing it with Eq. (12.15) reveals that in this case R_{\max} and K_M are both reduced by the factor, $\left(1 + \frac{[I]}{K_I} \right)$. Taking the reciprocal and simplifying Eq. (12.55) we get,

$$\frac{1}{R} = \frac{[S] + \frac{\left(\frac{k_{-1} + k_2}{k_1} \right)}{\left(1 + \frac{[I]}{K_I} \right)}}{k_2 [E]_0 [S]} = \frac{[S] \left(1 + \frac{[I]}{K_I} \right) + K_M}{R_{\max} [S]} \quad \dots (12.56)$$

Simplifying further, we get a familiar double reciprocal form as

$$\frac{1}{R} = \frac{K_M}{R_{\max}} \frac{1}{[S]} + \frac{1}{R_{\max}} \left(1 + \frac{[I]}{K_I} \right) \quad \dots (12.57)$$

Eq.(12.57) shows that a plot of $1/R$ versus $1/[S]$ will be linear with a slope of K_M / R_{\max} and an intercept of $1/R_{\max} (1 + [I] / K_I)$. A graphical representation of Lineweaver-Burk plot for the case of uncompetitive inhibition is given in Fig.12.9.

Contrary to the case of competitive inhibition, in this case of uncompetitive inhibition the slope is independent of the inhibitor concentration whereas the intercept depends on it. Therefore, for a series of concentrations of the inhibitors, a series of lines of identical slope (K_M / R_{max}) will be obtained. The intercepts on the other hand will depend on the inhibitor concentration.

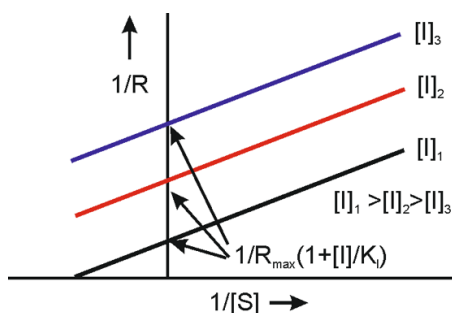
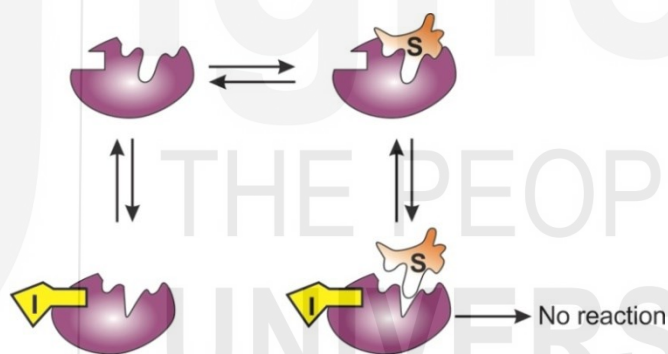


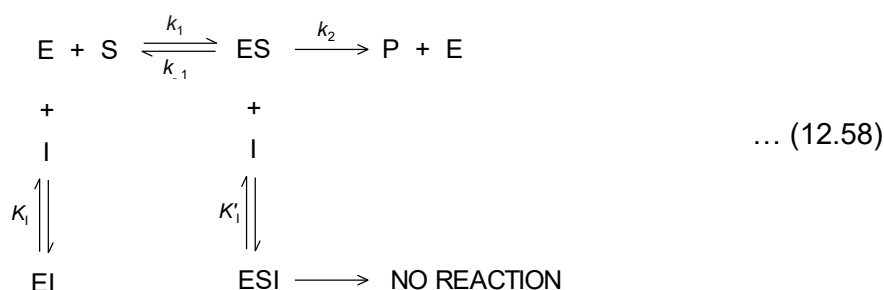
Fig. 12.9: A Lineweaver-Burk plot for the case of uncompetitive inhibition at three concentrations of inhibitor.

12.4.3 Non-Competitive Inhibition

In another case of inhibition the inhibitor binds to both E and ES to form EI and ESI respectively. It is assumed that ESI cannot break down to give product. This kind of inhibition is called **non-competitive (or mixed competitive) inhibition**. This type of inhibition can be shown pictorially as under.



The corresponding kinetic scheme can be represented as



In this case we have two separate equilibrium constants for the binding of the inhibitor to E and ES respectively. These need not be equal since it is quite likely that the presence of substrate will affect the ease with which the enzyme binds the inhibitor. If [S] is high (saturating) the substrate will knock off the inhibitor from EI complex and we have a situation similar to the uncompetitive case discussed above.

Proceeding in the same way as competitive and uncompetitive inhibitions, we can derive the following expression for the rate in case of non-competitive inhibition.

$$\frac{1}{R} = \frac{K_M}{R_{\max}} \left(1 + \frac{[I]}{K_I} \right) \frac{1}{[S]} + \frac{1}{R_{\max}} \left(1 + \frac{[I]}{K_I} \right) \quad \dots (12.59)$$

In this equation, K_I represents the combined effects of both K_I^{EI} and K_I^{ESI} .

This equation indicates that a plot of $1/R$ versus $1/[S]$ should be linear with a slope that represents $\frac{K_M}{R_{\max}} \left(1 + \frac{[I]}{K_I} \right)$ and an intercept equal to $\frac{1}{R_{\max}} \left(1 + \frac{[I]}{K_I} \right)$.

You may note that in this case, the slope of the line as well as the intercept both depend on the concentration of the inhibitor.

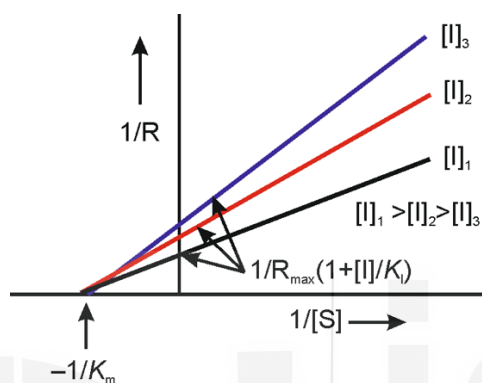


Fig. 12.10: A Lineweaver-Burk plot for the case of non-competitive enzyme inhibition at three concentrations of inhibitor.

A different line will be obtained for each initial concentration of the inhibitor $[I]$ used, but the lines have a common intercept of $1/K_m$ on the x-axis. This situation is illustrated graphically in Fig.12.10, and when this behaviour is observed, it is usually taken as a diagnostic test suggestive of noncompetitive inhibition.

Let us sum up what have you learnt in this unit. However, before that answer the following simple question to assess your understanding.

SAQ 5

In what way is the slope of line in the Lineweaver-Burk plot differ for competitive and uncompetitive inhibition?

12.5 SUMMARY

In this unit we discussed about the kinetics of enzyme catalysed reactions. We started the unit by discussing about enzymes, their characteristics, and the related terminology. We defined enzymes as biological catalysts that catalyse practically all chemical reactions occurring within a cell of living organisms. We described the nature of enzymes and recalled its important characteristics like, catalytic power, specificity, and regulation besides the mechanism of their action. This was followed by a discussion on the kinetics of enzyme catalysed reactions. Herein we explained the Michaelis–Menten mechanism and derived and analysed the corresponding mathematical expression for the rate of reaction. Thereafter, we took an important aspect of enzyme catalysis i.e.,

enzyme inhibition. Herein, we explained different types of enzyme inhibition mechanisms and discussed their kinetic characteristics and compared them with those of uninhibited reactions.

12.6 TERMINAL QUESTIONS

1. What are the effects of pH and temperature on enzyme activity?
2. Write the Michaelis-Menten equation and state the meaning of all the terms involved.
3. What is the difference between the Turnover Number (k_{cat}) and the Michaelis constant (K_M)?
4. List different mechanisms of enzyme inhibition.
5. Explain the difference between competitive and uncompetitive inhibition.
6. How does the presence of a competitive inhibitor affect the Michaelis-Menten equation and the Lineweaver-Burk plot?
7. What are the characteristic features of the Lineweaver-Burk plot for competitive, uncompetitive and non-competitive inhibitions?

12.7 ANSWERS

Self-Assessment Questions

1. Enzymes are biological catalysts that speed up chemical reactions in living organisms without themselves getting consumed. The three important characteristics of enzymes are:
 - Catalytic power
 - Specificity and
 - Regulation
2. According to Koshland's hypothesis on mechanism of enzyme action, the binding of the substrate to the enzyme causes alteration in the geometry of the enzyme such that the appropriate groups in the substrate and in the enzyme are in the correct orientation. It differs from the Fischer's hypothesis in that it considers the dynamic nature of enzyme (protein) whereas Fischer assumed the enzyme to have a fixed structure.
3. Lineweaver-Burk plot is an example of double reciprocal plot whereas Hanes-Wolf plot is a single reciprocal plot.
4. Lineweaver-Burk plot is a plot of $\frac{1}{R}$ versus the reciprocal of the substrate concentration, $1/[S]$. It is a straight line with slope of $\frac{K_M}{k_2[E]_0}$ and the intercept equal to $\frac{1}{k_2[E]_0}$. The reciprocal of the intercept gives the value of

R_{max} which can then be used along with the slope of the line to get the value of K_M .

5. In case of competitive inhibition the slope of line in the Lineweaver-Burk plot depends on the concentration of the inhibitor whereas in case of uncompetitive inhibition it remains unchanged on changing the concentration of the inhibitor.

Terminal Questions

1. The enzymes show their activity at optimum temperature and pH. Any variation (increase or decrease) in pH or the temperature would decrease the activity of the enzyme.
2. The simplified Michaelis-Menten equation is:

$$R = \frac{R_{max} [S]}{K_M + [S]}$$

The terms are as under:

R : the initial rate of the enzyme catalysed reaction

R_{max} : the maximum rate

$[S]$: substrate concentration

K_M : Michaelis constant

3. The turnover number of an enzyme is the number of substrate molecules or the moles of the substrate that is converted to product per second per mole of enzyme (or per mole of active site for a multi-subunit enzyme) when the enzyme is fully saturated with the substrate. On the other hand, Michaelis constant refers to the substrate concentration at which the initial rate of the enzyme catalysed reaction is one half of the maximum rate.
4. The different mechanisms of enzyme inhibition are:
- Competitive inhibition
 - Uncompetitive inhibition
 - Non-competitive inhibition
5. In competitive inhibition there is competition between the substrate, S, and the inhibitor, I, for the active site on the enzyme. On the other hand, in case of uncompetitive inhibition the inhibitor binds to the enzyme substrate complex and makes it unavailable for the product formation.