ENZYMES

NOMENCLATURE AND CLASSIFICATION

Enzymes are classified into six different groups according to the reaction being catalyzed. The nomenclature was determined by the Enzyme Commission in 1961 (with the latest update having occurred in 1992), hence all enzymes are assigned an “EC” number.

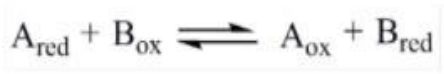
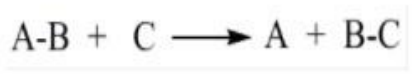
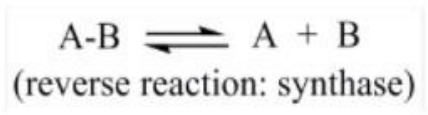
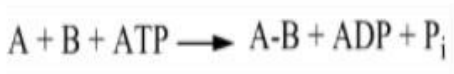
The nomenclature of enzymes is derived from their substrates or the catalyzed chemical reactions, and "ase" is usually added as a suffix. Enzymes can be indexed with letters and numbers according to International Union of Biochemistry and Molecular Biology: the letter EC plus four numbers representing four elements. The first number represents enzymes that are classified according to the mechanism of enzymatic reaction.

CLASSIFICATION

According to the unified classification principle of enzymes published by the International Society of Biochemistry, each group of enzymes in the above seven categories can be further divided into several subgroups according to the characteristics of the functional groups or bonds in the substrates. In order to show the properties of substrates or reactants more accurately, each subclass is further divided into subclasses and directly contains a quantity of enzymes.

Moreover, on the basis of the molecular composition, enzymes can be divided into pure enzymes and binding enzymes. Enzymes containing only protein are called pure enzymes. Binding enzymes are composed of proteins and cofactors. Only when the two components are combined, can the enzyme have catalytic activity.

OXIDOREDUCTASES, TRANSFERASES, HYDROLASES, LYASES, ISOMERASES, LIGASES, AND TRANSLOCASES. OXIDOREDUCTASES, TRANSFERASES AND HYDROLASES ARE THE MOST

Enzyme class	Reaction type	Description
EC 1 Oxidoreductases		Catalyze redox reaction and can be categorized into oxidase and reductase.
EC 2 Transferases		Catalyze the transfer or exchange of certain groups among some substrates
EC 3 Hydrolases		Accelerate the hydrolysis of substrates
EC 4 Lyases		Promote the removal of a group from the substrate to leave a double bond reaction or catalyze its reverse reaction
EC 5 Isomerases		Facilitate the conversion of isoisomers, geometric isomers or optical isomers.
EC 6 Ligases		Catalyze the synthesis of two molecular substrates into one molecular compound with the release energy
EC 7 Translocases		Catalyze the movement of ions or molecules across membranes or their separation within membranes

A BRIEF GUIDE TO ENZYME NOMENCLATURE AND CLASSIFICATION

NC-IUBMB Enzyme List, or, to give it its full title, “Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes by the Reactions they Catalyse,

1 is a functional system, based solely on the substrates transformed and products formed by an enzyme. The basic layout of the classification for each enzyme is described below with some indication of the guidelines followed. More detailed rules for enzyme nomenclature and classification are available online.

2 Further details of the principles governing the nomenclature of individualenzyme classes are given in the following sections.

2. Basic Concepts .

EC numbers

Enzymes are identified by EC (Enzyme Commission) numbers. These are also valuable for relating the information to other databases. They were divided into 6 major classes according to the type of reaction catalysed and a seventh, the translocases, was added in 2018.

3 These are

Table 1. Enzyme classes

Name Reaction catalysed

1 Oxidoreductases *AH2 + B = A +BH2
2 Transferases AX + B = BX + A
3 Hydrolases A-B + H2O = AH + BOH
4 LyasesA=B + X-Y = A-B ç ç
X Y
5 Isomerases A = B
6 Ligases †
A + B + NTP = A-B + NDP + P (or NMP + PP)
7 Translocases AX + B çç = A + X + ççB (side 1) (side 2)

*Where nicotinamide-adenine dinucleotides are the acceptors, NAD+ and NADH + H+ are used, by convention.

The EC number is made up of four components separated by full stops. The first identifies the class of reaction catalysed. The second number (the subclass) generally contains information about the type of compound or group involved. For the oxidoreductases, the subclass indicates the type of group in the donor that undergoes oxidation or reduction (e.g., 1.1. acts on the CHOH group of donors whereas

1.4. acts on the CH-NH2 group of donors). New subclasses may be created as new information

or interpretations become available, e.g., a new subclass, EC 5.6: ‘ Isomerases altering

macromolecular conformation’ has recently been added to the Isomerases

class.

The third number, the sub-subclass, further specifies the type of reaction involved. For instance, EC 1.x.1.- indicates that NAD+ or NADP+ is the acceptor, while 1.x.2.- has a cytochrome as the acceptor, etc. The fourth is a serial number that is used to identify the individual enzyme within a sub-subclassA list of the numbers for different enzyme classes etc. can be found online.

EC 1 [+] **Oxidoreductases**
EC 2 [+] **Transferases**
EC 3 [+] **Hydrolases**
EC 4 [+] **Lyases**
EC 5 [+] **Isomerases**
EC 6 [–] **Ligases**
- EC 6.1 [+] Forming carbon-oxygen bonds
- EC 6.2 [+] Forming carbon-sulfur bonds
- EC 6.3 [–] Forming carbon-nitrogen bonds
  - EC 6.3.1 [+] Acid—ammonia (or amine) ligases (amide synthases)
  - EC 6.3.2 [+] Acid—amino-acid ligases (peptide synthases)
  - EC 6.3.3 [+] Cyclo-ligases
  - EC 6.3.4 [+] Other carbon-nitrogen ligases
  - EC 6.3.5 [+] Carbon-nitrogen ligases with glutamine as amido-N-donor
- EC 6.4 [+] Forming carbon-carbon bonds
- EC 6.5 [+] Forming phosphoric-ester bonds
- EC 6.6 [+] Forming nitrogen—metal bonds

EC 7 [+] **Translocases**

specificity of enzyme action

Specificity is the ability of an enzyme to choose exact substrate from a group of similar chemical molecules. The specificity is actually a molecular recognition mechanism and it operates through the structural and conformational complementarity between enzyme and substrate.

One of the properties of enzymes that makes them so important as diagnostic and research tools is the specificity they exhibit relative to the reactions they catalyze. A few enzymes exhibit absolute specificity; that is, they will catalyze only one particular reaction. Other enzymes will be specific for a particular type of chemical bond or functional group. In general, there are four distinct types of specificity:

Absolute specificity - the enzyme will catalyze only one reaction.

Group specificity - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.

Linkage specificity - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.

Stereochemical specificity - the enzyme will act on a particular steric or optical isomer.

Though enzymes exhibit great degrees of specificity, cofactors may serve many apoenzymes. For example, nicotinamide adenine dinucleotide (NAD) is a coenzyme for a great number of dehydrogenase reactions in which it acts as a hydrogen acceptor. Among them are the alcohol dehydrogenase, malate dehydrogenase and lactate dehydrogenase reactions.

Enzymes show different degrees of specificity:

1. Relative, low or bond specificity

In this type the enzyme acts on substrates that are similar in structure and contain the same type of bonds e.g.

a. Amylase, which acts on α 1-4 glycosidic, bonds in starch, dextrin and glycogen.
b. Lipase that hydrolyzes ester bonds in different triglycerides

2. Moderate, structural or group specificity

In this type of specificity, the enzyme is specific not only to the type of bond but also to the structure surrounding it.

For example:

a. **Pepsin** is an endopeptidase that hydrolyzes central peptide bonds in which the amino group belongs to aromatic amino acids e.g. phenyl alanine, tyrosine and tryptophan.

b. **Trypsin** is an endopeptidase that hydrolyzes central peptide bonds in which the amino group belongs to basic amino acids e.g. arginine, lysine and histidine.

c. **Chymotrypsin** is an endopeptidase that hydrolyzes central peptide bonds in which the carboxyl group belongs to aromatic amino acids.

d. **Aminopeptidase** is an exopeptidase that hydrolyzes peripheral peptide bond at the amino terminal (end) of polypeptide chain.

e. **Carboxypeptidase** is an exopeptidase that hydrolyzes peripheral peptide bond at the carboxyl terminal of polypeptide chain.

3. Absolute, high or substrate specificity

In this type of specificity, the enzyme acts only on one substrate e.g.

a) Uricase, which acts only on uric acid.
b) Arginase, which acts only on arginine.
c) Carbonic anhydrase, which acts only on carbonic acid.
d) Lactase, which acts on lactose.
e) Sucrase, which acts on sucrose.
f) Maltase, which acts on maltose.

4. Optical or stereo-specificity

In this type of specificity, the enzyme is specific not only to the substrate but also to its optical configuration e.g.

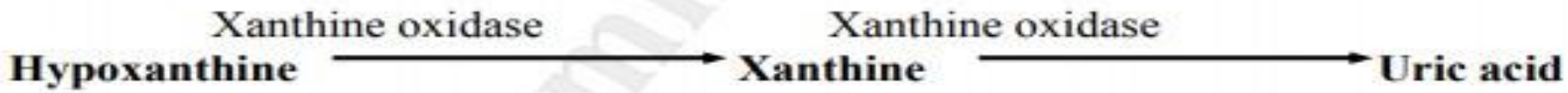
- **a.** L amino acid oxidase acts only on L amino acids.
- **b.** D amino acid oxidase acts only on D amino acids.
- **c.** α- glycosidase acts only on α- glycosidic bonds, which are present in starch, dextrin and glycogen.
- **d.** β- glycosidase acts only on β- glycosidic bonds that are present in cellulose.

N.B. We can digest glycogen and starch due to presence of α-glycosidase, but we can not digest cellulose due to the absence of β-glycosidase

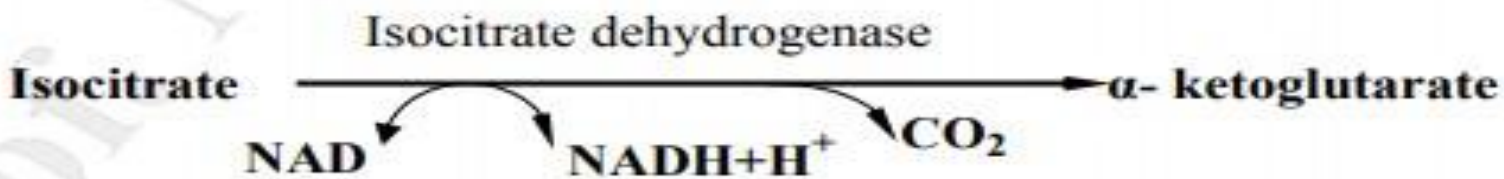
5. Dual specificity

There are two types of dual specificity:

A- The enzyme may act on two substrates by one reaction type. e.g. xanthine oxidase enzyme acts on xanthine and hypoxanthine (two substrates) by oxidation (one reaction type).



B- The enzyme may act on one substrate by two different reaction types e.g. isocitrate dehydrogenase enzyme acts on isocitrate (one substrate) by oxidation followed by decarboxylation (two different reaction types).



ISOZYME

- **Isozymes (also known as isoenzymes or more generally as multiple forms of enzymes) are enzymes that differ in amino acid sequence but catalyze the same chemical reaction. These enzymes usually display different kinetic parameters (e.g. different KM values), or different regulatory properties. The existence of isozymes permits the fine-tuning of metabolism to meet the particular needs of a given tissue or developmental stage. In biochemistry, isozymes (or isoenzymes) are isoforms (closely related variants) of enzymes. In many cases, they are coded for by homologous genes that have diverged over time. Although, strictly speaking, allozymes represent enzymes from different alleles of the same gene, and isozymes represent enzymes from different genes that process or catalyse the same reaction, the two words are usually used interchangeably.**

Introduction

Isozymes were first described by R. L. Hunter and Clement Markert (1957) who defined them as different variants of the same enzyme having identical functions and present in the same individual.

This definition encompasses (1) enzyme variants that are the product of different genes and thus represent different loci (described as isozymes) and

(2) enzymes that are the product of different alleles of the same gene (described as allozymes).

Isozymes are usually the result of gene duplication, but can also arise from polyploidisation or nucleic acid hybridization. Over evolutionary time, if the function of the new variant remains identical to the original, then it is likely that one or the other will be lost as mutations accumulate, resulting in a pseudogene. However, if the mutations do not immediately prevent the enzyme from functioning, but instead modify either its function, or its pattern of expression, then the two variants may both be favoured by natural selection and become specialised to different functions.

For example, they may be expressed at different stages of development or in different tissues.

Allozymes may result from point mutations or from insertion-deletion (indel) events that affect the coding sequence of the gene. As with any other new mutations, there are three things that may happen to a new allozyme:

It is most likely that the new allele will be non-functional—in which case it will probably result in low fitness and be removed from the population by natural selection.

Alternatively, if the amino acid residue that is changed is in a relatively unimportant part of the enzyme (e.g., a long way from the active site), then the mutation may be selectively neutral and subject to genetic drift.

In rare cases, the mutation may result in an enzyme that is more efficient, or one that can catalyse a slightly different chemical reaction, in which case the mutation may cause an increase in fitness, and be favoured by natural selection

Mechanisms of enzyme action

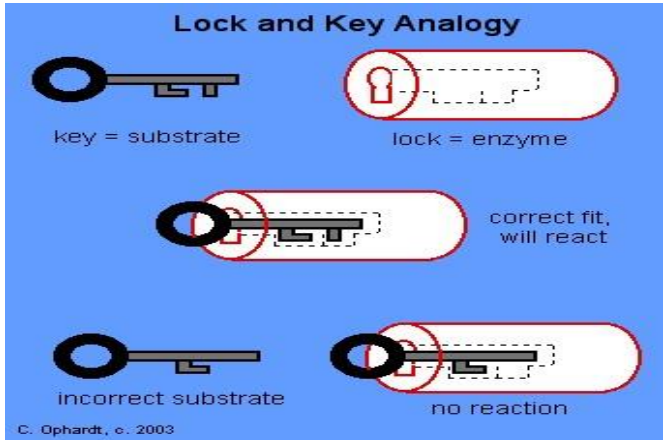
Introduction - Enzyme Characteristics:

The basic mechanism by which enzymes catalyze chemical reactions begins with the binding of the substrate (or substrates) to the active site on the enzyme. The active site is the specific region of the enzyme which combines with the substrate. The binding of the substrate to the enzyme causes changes in the distribution of electrons in the chemical bonds of the substrate and ultimately causes the reactions that lead to the formation of products. The products are released from the enzyme surface to regenerate the enzyme for another reaction cycle. The active site has a unique geometric shape that is complementary to the geometric shape of a substrate molecule, similar to the fit of puzzle pieces. This means that enzymes specifically react with only one or a very few similar compounds.

Lock and Key Theory:

The specific action of an enzyme with a single substrate can be explained using a Lock and Key analogy first postulated in 1894 by Emil Fischer. In this analogy, the lock is the enzyme and the key is the substrate. Only the correctly sized key (substrate) fits into the key hole (active site) of the lock (enzyme).

Smaller keys, larger keys, or incorrectly positioned teeth on keys (incorrectly shaped or sized substrate molecules) do not fit into the lock (enzyme). Only the correctly shaped key opens a particular lock. This is illustrated in graphic on the left.

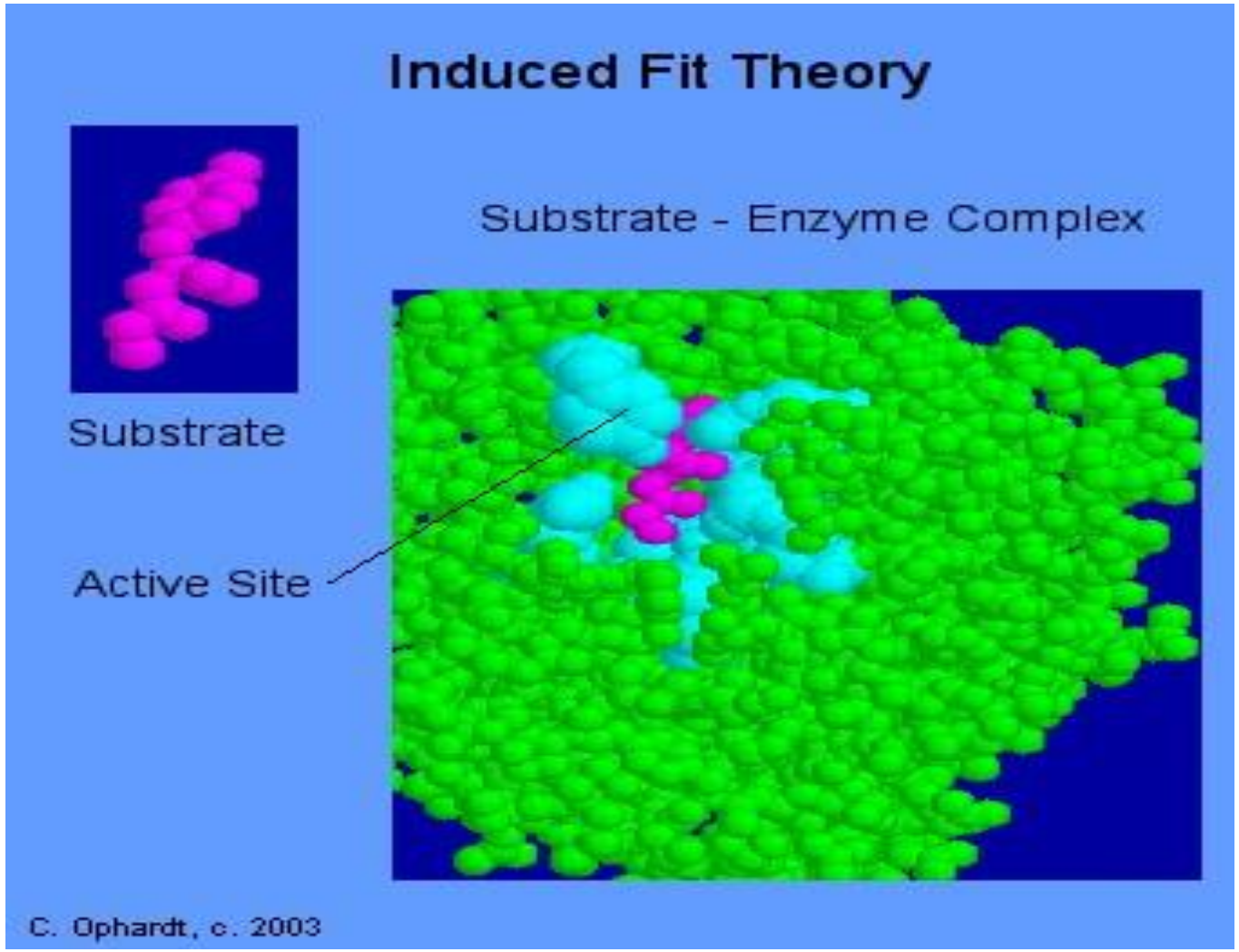


Induced Fit Theory:

Not all experimental evidence can be adequately explained by using the so-called rigid enzyme model assumed by the lock and key theory. For this reason, a modification called the inducedfit theory has been proposed.

The induced-fit theory assumes that the substrate plays a role in determining the final shape of the enzyme and that the enzyme is partially flexible. This explains why certain compounds can bind to the enzyme but do not react because the enzyme has been distorted too much. Other molecules may be too small to induce the proper alignment and therefore cannot react. Only the proper substrate is capable of inducing the proper alignment of the active site.

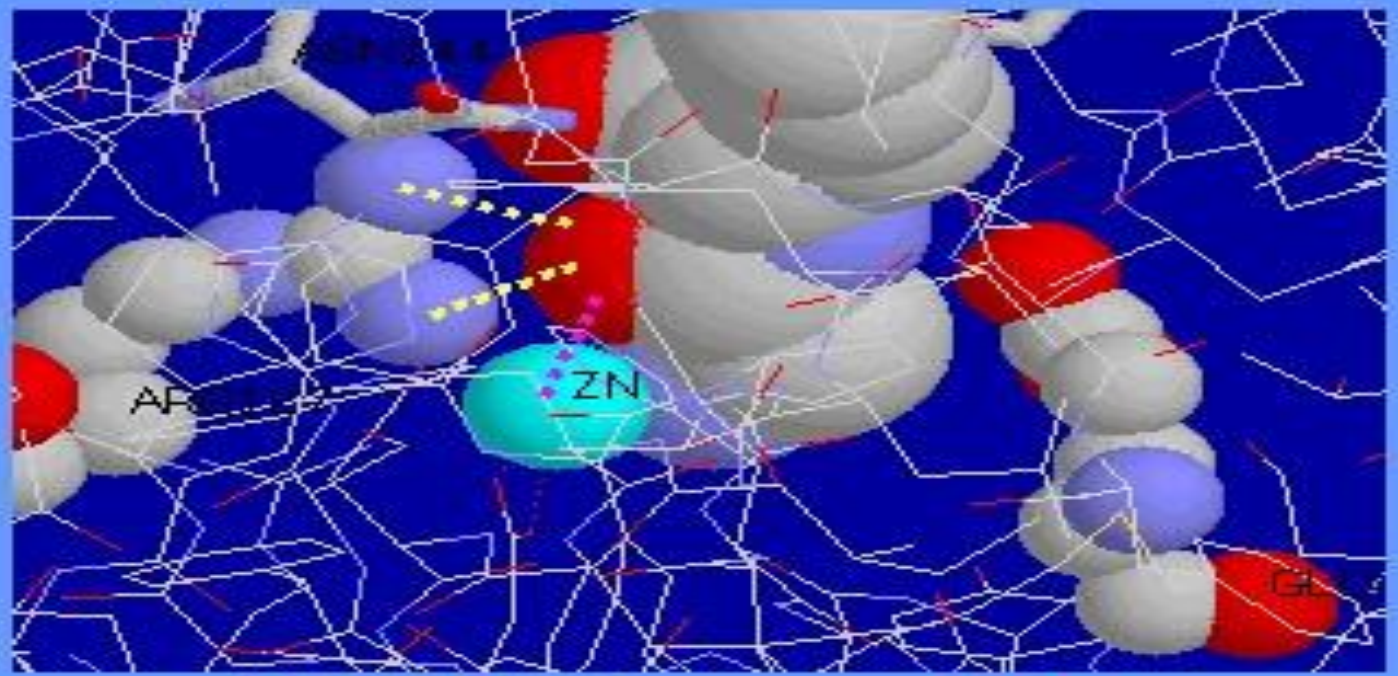
In the graphic on the left, the substrate is represented by the magenta molecule, the enzyme protein is represented by the green and cyan colors. The cyan colored protein is used to more sharply define the active site. The protein chains are flexible and fit around the substrate.


Induced Fit Theory
Substrate - Enzyme Complex
Substrate
Active Site
C. Ophardt, c. 2003

Nature of Active Site and Substrate Interaction: Enzymes have varying degrees of specificity. Some enzymes have absolute specificity for one substrate and no others, while other enzymes react with substrates with similar functional groups, side chains, or positions on a chain. The least specific enzymes catalyze a reaction at a particular chemical bond regardless of other structural features.

Much experimental work is devoted to gaining an understanding of the nature of the active site in an enzyme. Since enzymes are proteins, the nature of amino acid side chains in the vicinity of the active site is important. The specific amino acid side chains have been determined for many enzymes. The active site for carboxypeptidase A will be used to illustrate the principles involved as shown in the graphic on the left.

Active Site Interactions



Hydrogen bonds are shown in yellow

Zinc ion coordination bond in magenta

C. Ophardt, c. 2003

The substrate (space filling gray,blue red) can interact with the active site through opposite charges, hydrogen bonding (shown in yellow), hydrophobic non-polar interaction, and coordinate covalent bonding to the metal ion activator as shown in magenta. The numbers behind the amino acids indicate the sequence position of the amino acid in the protein. The white lines represent the wire frames of the other amino acids in the
enzyme.

The carbonyl bond is activated by interaction with the Zn ions. This leads to the addition of -OH from water to the carbonyl to produce an acid and the ultimate rupture of the C-
N bond.

Enzyme kinetics is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated.

ENZYMES KINETICS

Kinetics is the study of reaction rates. This will be considered in the context of enzymes where the rate of the reaction means the rate of product formation. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

Enzyme Function

Enzymes lower the activation energy, Ea, of a particular reaction. They can do this because they have a high affinity for a transition state. The activation energy is the minimum energy needed for a reaction to occur. Enzymes assist in the reaction so that less energy is needed. This means the reaction can occur more easily. This speeds up the rate of the reaction as it allows the product to be formed faster.

An enzyme has a high affinity for the transition state (even higher than for its substrate). Therefore when the substrate binds, it is quickly forced into the transition state. This is a state that exists between the substrate and the product. The enzyme is said to facilitate the formation of the transition state.

The transition state has a high energy, making it very unstable. It can only exist transiently.

The transition state spontaneously turns into the more stable product with lower energy.

The enzyme will have a low affinity for the product and so the product is released.

Rate Limiting Steps

The rate limiting step in any reaction is its slowest step. It sets the pace for the entire reaction. After all, a production line can only be as productive as its slowest worker. In enzymatic reactions, the conversion of the enzyme-substrate complex to the product is normally rate limiting. The rate of this step (and therefore the entire enzymatic reaction) is directly proportional to the concentration of ES.

The concentration of ES changes as the reaction progresses. Therefore, the rate of product formation also changes over time. When the reaction reaches equilibrium (steady state) the concentration of ES (and therefore the rate) remains relatively constant.

Reaction Kinetics

When an enzyme is added to a lot of substrate, the reaction that follows occurs in three stages with distinct kinetics:

Phase	Concentration of ES	Rate of product formation

Pre-steady state	**Burst** of ES complexes form	**Slow** as must first wait for ES to form. Speeds up as ES forms.
Steady state(equilibrium)	ES remains **constant**. It is formed as quickly as it breaks down.	**Constant** rate of formation. Faster than pre-steady state.
Post-steady state	Substrate **depletes** so fewer ES complexes can form	**Slow** as there are fewer ES complexes. Slows down as substrate runs out.

The pre-steady state phase is very short as equilibrium is reached within microseconds. If you measure the rate in the first few seconds of a reaction (V0) you are actually measuring the steady state. This is the rate used in Michaelis-Menten Kinetics.

Michaelis-Menten Kinetics

Two terms that are important within Michaelis-Menten Kinetics are:

Vmax – the maximum rate of reaction when all enzyme active sites are saturated with substrate

Km – the substrate concentration that gives half maximal velocity. Km is a measure of the affinity an enzyme has for its substrate, as a lower Km means that less of the substrate is required to reach half of Vmax.

This equation concerns the steady state of an enzymatic reaction with one substrate, and is given by:

$$v = \frac{V_{max}[S]}{K_M + [S]}$$

It describes how the initial rate of reaction, V0, is affected by the initial substrate concentration, [S]0. It only looks at the start of the reaction. This allows it to ignore the reverse reaction where substrate is formed from product. This is because at the start of the reaction there is no product present to become substrate.

The plot of rate against concentration has the shape of a rectangular hyperbola. However, a more useful representation of Michaelis–Menten kinetics is a graph called a

Lineweaver–Burk plot. The equation used to generate this plot is given by:

$$\frac{1}{V} = \frac{K_m}{V_{max}}\frac{1}{S} + \frac{1}{V_{max}}$$

This allows an easier interpretation of various quantities from the graph, such as the presence of an inhibitor. This is shown in figure 3, where an inhibitor decreases Km and therefore shifts the original line upwards.

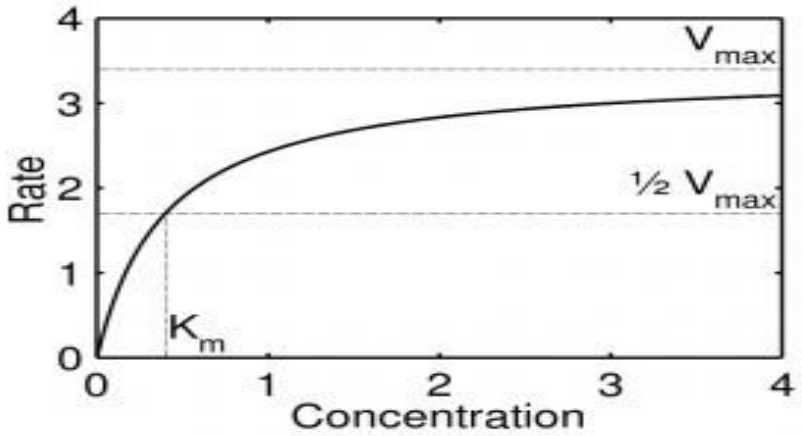


Fig – image of an enzyme displaying Michaelis–Menten kinetics.

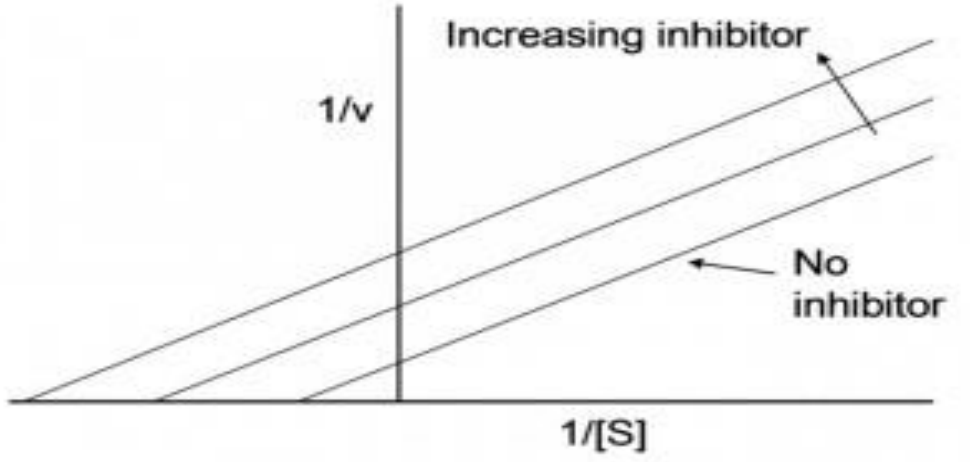


Fig – Image of a Lineweaver–Burk plot

Factors affecting rate of enzyme catalyze reaction

1. Effect of enzyme concentration

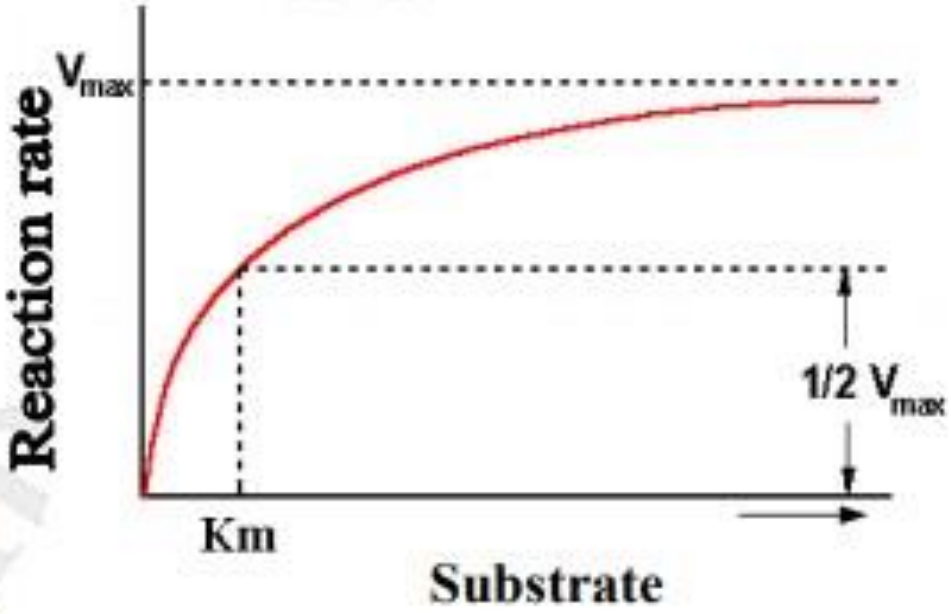
The rate of enzyme action is directly proportional to the concentration of enzyme provided that the condition of the reaction remains constant and sufficient substrate is supplied.

2. Effect of substrate concentration

The rate of reaction increases as the substrate concentration increases until a certain point (Vmax) at which the reaction attains maximal velocity.

Any increase in substrate concentration after this point does not cause further increase in the rate of the reaction because at Vmax enzyme molecules are completely saturated with substrate molecules.

The substrate concentration that causes the reaction to proceed at its half maximal velocity (1/2 Vmax) is called Michaelis constant (Km). Enzymes that have low Km have high affinity to

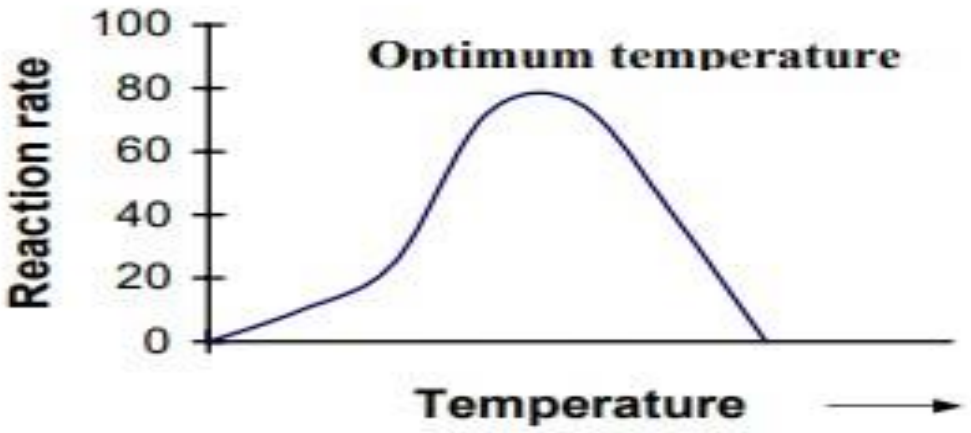


Factors affecting rate of enzyme catalyze reaction

the substrate and act at maximal velocity at low substrate concentration e.g. hexokinase enzyme that acts on glucose in the fasting state (low glucose concentration).
Enzymes with high Km have low affinity to substrate and need high concentration of substrates e.g. glucokinase which needs high concentration of glucose so it acts maximally in the fed state.

Effect of temperature

At very low temperature, enzymes are inactive. Enzyme activity increases gradually with the rise in temperature until a temperature at which the enzyme attains its maximal activity, this temperature is called optimum temperature, which lies between 37 – 40 °C in humans.
Optimum temperature is the temperature at which the enzyme attains its maximal activity. The rise in temperature from low temperature to optimum temperature causes an increase in the rate of reaction due to:



a. The rise in temperature increases the initial energy of substrate leading to a decrease in the activation energy and lower the energy barrier of the reaction.

Factors affecting rate of enzyme catalyze reaction

b. Also, the rise in temperature increases collision of the molecules i.e. more molecules become in the bond forming or bond breaking distance.

The rise in temperature above the optimum temperature leads to a decrease in the rate of enzyme activity.

At higher temperature (60 – 65 °C in humans) irreversible loss of enzyme activity occurs due to denaturation of the enzymes, which are protein in nature.

4. Effect of pH

Each enzyme has an optimum pH at which it attains its maximal activity e.g.

a. Optimum pH of pepsin is 1.5 – 2 (acidic).

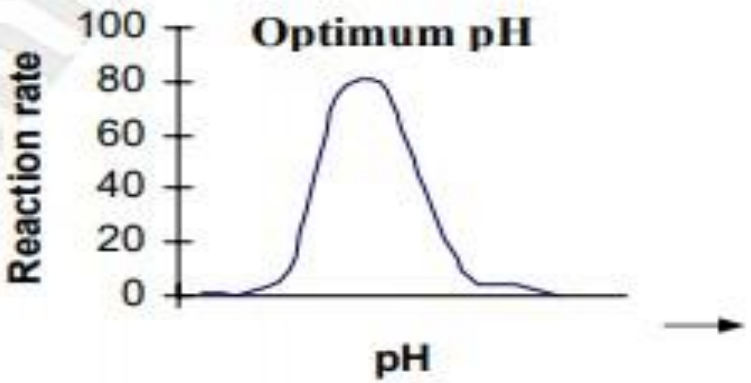
b. Optimum pH of pancreatic lipase is 7.5 – 8 (alkaline).

c. Optimum pH of salivary amylase is 6.8 (slightly acidic).

Any change of pH below or above the optimum pH decreases the rate of enzyme action due to:

a. Changes in pH lead to changes in the ionization state of the substrate or the enzyme or both.

b. Also, extreme changes in pH lead to denaturation of the enzyme that is protein in



5. Effect of time

At the beginning, the rate of reaction increases but by time the rate of reaction decreases due to:

a. Depletion of substrate.
b. Accumulation of end products.
c. Change in pH of the reaction, which becomes different from the optimum pH of the enzyme.

6. Concentration of coenzymes

In the conjugated protein enzymes that need coenzymes, the increase in the coenzyme concentration causes an increase in the rate of enzyme action.

7. Concentration of metal ion activators

The increase in metal ion activators increases the rate of enzyme action. Many enzymes are activated by metal ions e.g.

a. Chloride ions activate salivary amylase.
b. Calcium ions activate thrombokinase enzyme.

8. Presence of inhibitors

Inhibitors decrease or even abolish enzyme activity.
Enzyme inhibitors may be:

a. Competitive inhibitors
b. Noncompetitive inhibitors

MULTI-SUBSTRATE REACTION

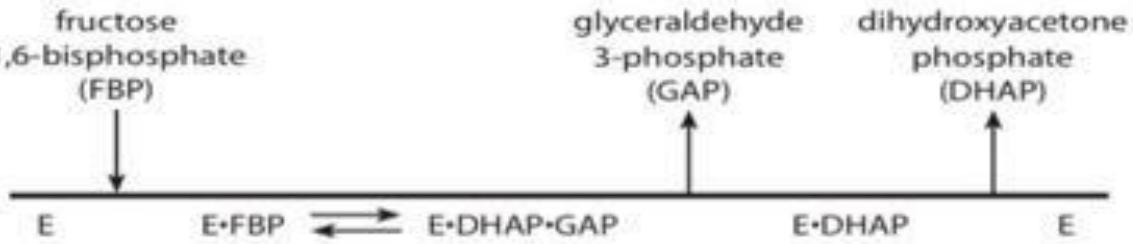
Multi-substrate reactions. Multi-substrate reactions follow complex rate equations that describe how the substrates bind and in what sequence. ... For an enzyme that takes two substrates A and B and turns them into two products P and Q, there are two types of mechanism: ternary complex and ping–pong.

Multiple-substrate Reactions:

- Involve more than one substrate often resulting in multiple products.
- Known as bi bi reactions (two substrates two products one enzyme)
- The following equation represents a bi bi reaction in which A and B are the substrates, E is the enzyme and P, Q are the products (considering the forward direction).
- 

- Overall reaction does not give a good representation of what actually happens in reality.
- The Multi-Substrate reactions in fact, follow a complex create equations that describe how the substrates bind and in what sequence.
- More information about the enzyme-reagent interaction is obtained when looking at the enzyme mechanism which consists of microscopic reactions

1. A common notation for the depiction of binding events in a multi-substrate enzyme is to show the enzyme as a horizontal line with arrows showing the binding or release of reactants and products. For example, the reaction catalyzed by Zn^{2+}-dependent aldolase is depicted in the following diagram:



The reaction catalyzed by Schiff-base forming aldolase, on the other hand, is depicted:

a. Diagram the reaction catalyzed by hexokinase if glucose must bind before ATP and ADP must dissociate before glucose 6-phosphate.

b. Diagram the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase.

Determination of Kinetic Parameters in Multi-Substrate Systems

* With one substrate variable and the other(s) fixed:

-- generate plots kinetics plots that can be analyzed used any of the graphical or computational tools that we discussed with single substrate enzymes.

* BUT, V & Km parameters derived are apparent values only. What do they mean?

-- change in conc. of fixed substrate(s) will change apparent V & Km for variable one. *

To get true values, we have to extend our definitions of V & Km a bit from those we used for single substrate systems.

FOR MULTIPLE SUBSTRATES:

(1) Maximal velocity, V, is defined as the reaction velocity which occurs when all substrates are at saturation levels.

(2) Each substrate will have its own Michaelis constant which is defined as the concentration of that substrate which gives a velocity of half the maximal velocity when all other substrates are present at saturation levels. * Analysis methods are also extensions of methods used for single substrate systems.

4 Use of Lineweaver-Burk Plot to Estimate "True" Multi-Substrate Kinetic Param

Primary LB plot for sequential enzyme

A + B <=> P + Q A is variable, B is fixed

Secondary Plot 1/Vapp for different

fixed [B]'s -1/KA, app
for different fixed [B]'s
true

* Likewise, for primary plot with B variable and A fixed, a secondary plot of 1/Vapp vs 1/[A]:

(1) gives -1/KA,true from the horizontal intercept;

(2) also gives 1/Vapp from the vertical intercept, which should equal the value above.

5 Deriving Rate Equations: King-Altman Method Ordered Sequential BiBi Mechanism:

All rate constants must be first-order;

e.g. the second-order rate constant k+1 must be represented by a pseudo-firstorder constant by including the concentration of A: k+1a

A master pattern is drawn representing the skeleton of the scheme; here a square:

Now find every pattern that: (1) consists only of lines from the master pattern;

(2) connects every enzyme species; and

(3) contains no closed loops.

YES: NO:

Next, for each enzyme species, draw arrows on each

pattern, leading to the species considered,

regardless of starting point. Thus for E:

Then a sum of products of rate constants is written, such that each product contains the

rate constants corresponding to the arrows. So, from the patterns leading to E, the sum of

products is:

k-1k-2k-3p + k-1k-2k+4 + k-1k+3k+4 + k+2k+3k+4b

This sum is then the numerator of an expression representing the fraction of the total enzyme concentration

e0 present as the species in question. So for all four species we have:

The denominator S is the sum of all 4 numerators, i.e. the sum of all 16 products obtained from the pattern.

The rate of the reaction is then the sum of the rates of steps that generate one particular

product, minus the sum of the rates of steps that consume the same product. In this

example, there is only one step that generates P: (EAB + EAQ) --> EQ + P, and only one step that consumes P: EQ + P --> (EAB + EPQ), so we have

GENERAL RULE FOR NUMERATOR:

-- Positive term is the product of total enzyme conc., all substrate concentrations for the forward rxn, and all rate constants for a complete cycle in the forward direction. -- Negative term is the product of total enzyme conc., all substrate concentrations for the reverse rxn, and all rate constants for a complete cycle in the reverse direction.

9 “For most purposes it is more important to know the form of the steady-state rate equation than to know its detailed expression in terms of rate constants.” --A. Cornish-Bowden

COEFFICIENT FORM (Ordered Sequential BiBi)

ENZYME INHIBITION.

An enzyme inhibitor is a molecule that binds to an enzyme and decreases its activity. By binding to enzymes' active sites, inhibitors reduce the compatibility of substrate and enzyme and this leads to the inhibition of Enzyme-Substrate complexes' formation, preventing the catalyzation of reactions and decreasing (at times to zero) the amount of product produced by a reaction. It can be said that as the concentration of enzyme inhibitors increases, the rate of enzyme activity decreases, and thus, the amount of product produced is inversely proportional to the concentration of inhibitor molecules

The binding of an inhibitor can stop a substrate from entering the enzyme's active site and/or hinder the enzyme from catalyzing its reaction. Inhibitor binding is either reversible or irreversible. Irreversible inhibitors usually react with the enzyme and change it chemically (e.g. via covalent bond formation). These inhibitors modify key amino acid residues needed for enzymatic activity. In contrast, reversible inhibitors bind non-covalently and different types of inhibition are produced depending on whether these inhibitors bind to the enzyme, the enzyme-substrate complex, or both.

Many drug molecules are enzyme inhibitors, so their discovery and improvement is an active area of research in biochemistry and pharmacology. A medicinal enzyme inhibitor is often judged by its specificity (its lack of binding to other

proteins) and its potency (its dissociation constant, which indicates the concentration needed to inhibit the enzyme). A high specificity and potency ensure that a drug will have few side effects and thus low toxicity.

Enzyme inhibitors also occur naturally and are involved in the regulation of metabolism. For example, enzymes in a metabolic pathway can be inhibited by downstream products. This type of negative feedback slows the production line when products begin to build up and is an important way to maintain homeostasis in a cell.

1.Reversible inhibitors

1.1 Types of reversible inhibitors

1.2 Quantitative description of reversible inhibition

1.3 Measuring the dissociation constants of a reversible inhibitor

1.4 Reversible inhibitors

1.5 Special cases

1.6 Examples of reversible inhibitors

2 Irreversible inhibitors

2.1 Types of irreversible inhibition (covalent inactivation)

2.2 Analysis of irreversible inhibition

2.3 Special cases

REVERSIBLE INHIBITORS

Reversible inhibitors attach to enzymes with non-covalent interactions such as hydrogen bonds, hydrophobic interactions and ionic bonds. Multiple weak bonds between the inhibitor and the active site combine to produce strong and specific binding. In contrast to substrates and irreversible inhibitors, reversible inhibitors generally do not undergo chemical reactions when bound to the enzyme and can be easily removed by dilution or dialysis.

There are four kinds of reversible enzyme inhibitors. They are classified according to the effect of varying the concentration of the enzyme's substrate on the inhibitor.

In competitive inhibition,
the substrate and inhibitor cannot bind to the enzyme at the same time, as shown in the figure on the right. This usually results from the inhibitor having an affinity for the active site of an enzyme where the substrate also binds; the substrate and inhibitor compete for access to the enzyme's active site. This type of inhibition can be overcome by sufficiently high concentrations of substrate (Vmax remains constant), i.e., by out-competing the inhibitor. However, the apparent Km will increase as it takes a higher concentration of the substrate to reach the Km point, or half the Vmax. Competitive inhibitors are often similar in structure to the real substrate (see examples below).

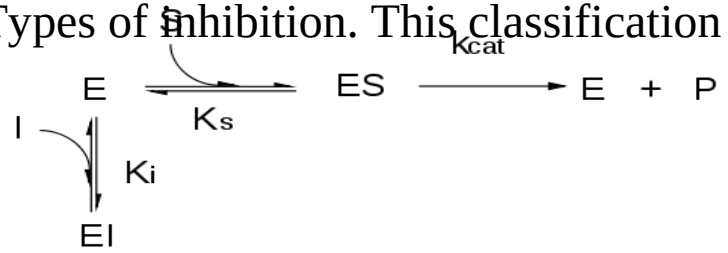
In uncompetitive inhibition, the inhibitor binds only to the substrate-enzyme complex. This type of inhibition causes Vmax to decrease (maximum velocity decreases as a result of removing activated complex) and Km to decrease (due to better binding efficiency as a result of Le Chatelier's principle and the effective elimination of the ES complex thus decreasing the Km which indicates a higher binding affinity).

In non-competitive inhibition, the binding of the inhibitor to the enzyme reduces its activity but does not affect the binding of substrate. As a result, the extent of inhibition depends only on the concentration of the inhibitor. Vmax will decrease due to

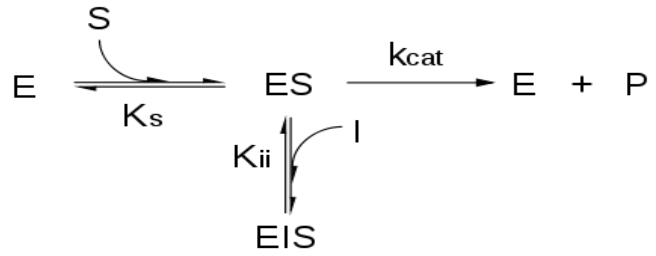
the inability for the reaction to proceed as efficiently, but Km will remain the same as the actual binding of the substrate, by definition, will still function properly.

In mixed inhibition, the inhibitor can bind to the enzyme at the same time as the enzyme's substrate. However, the binding of the inhibitor affects the binding of the substrate, and vice versa. This type of inhibition can be reduced, but not overcome by increasing concentrations of substrate. Although it is possible for mixed-type inhibitors to bind in the active site, this type of inhibition generally results from an allosteric effect where the inhibitor binds to a different site on an enzyme. Inhibitor binding to this allosteric site changes the conformation (i.e., tertiary structure or three-dimensional shape) of the enzyme so that the affinity of the substrate for the active site is reduced.

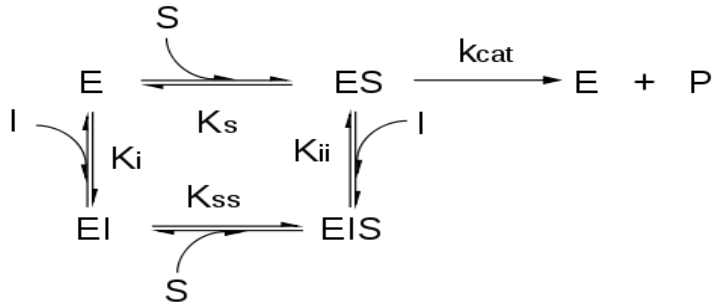
Types of inhibition. This classification was introduced by W.W. Cleland.[3]



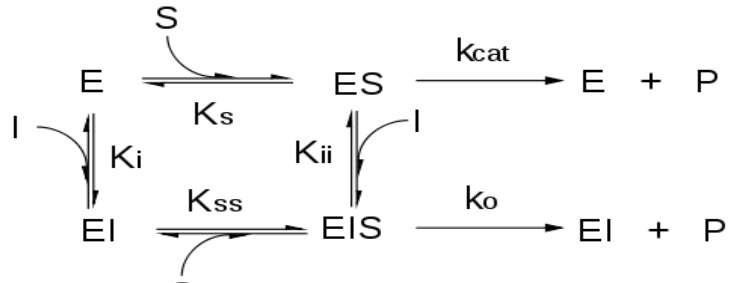
competitive inhibition



uncompetitive inhibition



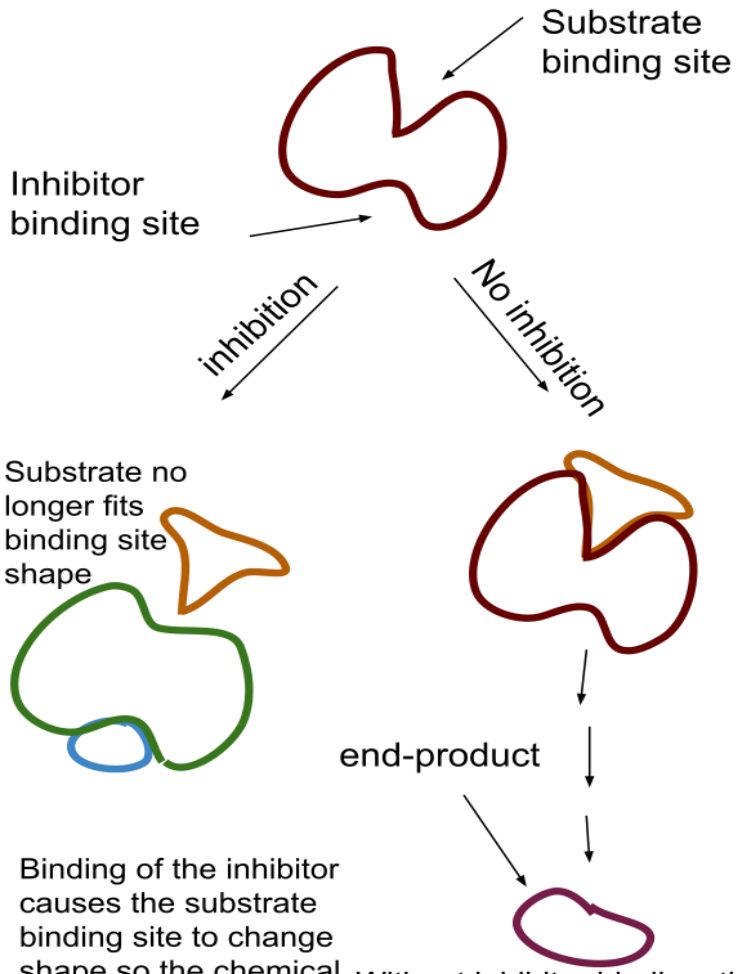
non-competitive inhibition



mixed inhibition

A figure comparing the three types of enzyme inhibitors and how they work in regards to substrate binding sites and inhibitors binding sites.

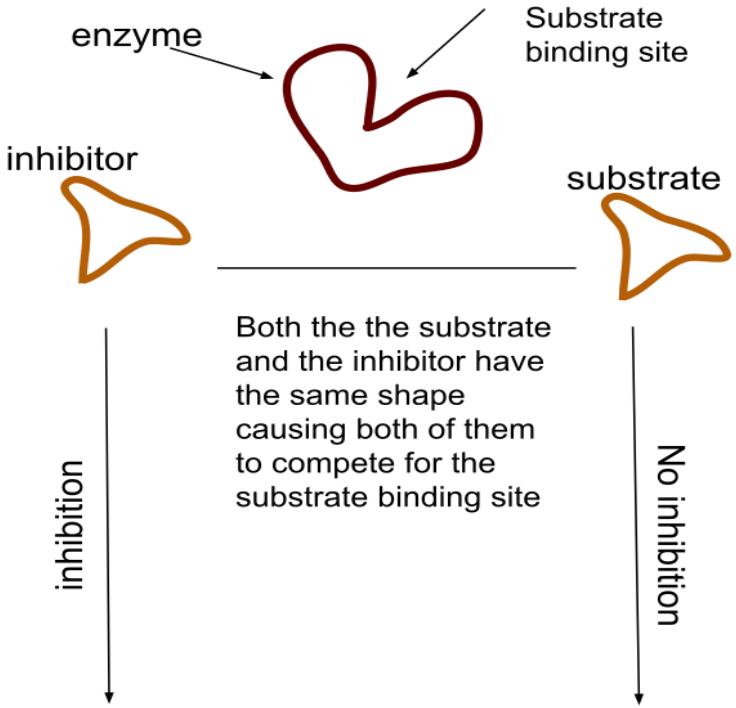
NON-COMPETITIVE INHIBITION



Binding of the inhibitor causes the substrate binding site to change shape so the chemical reaction is not carried out

Without inhibitor binding, the substrate can bind causing the chemical reaction to be carried out and for the end

COMPETITIVE INHIBITION

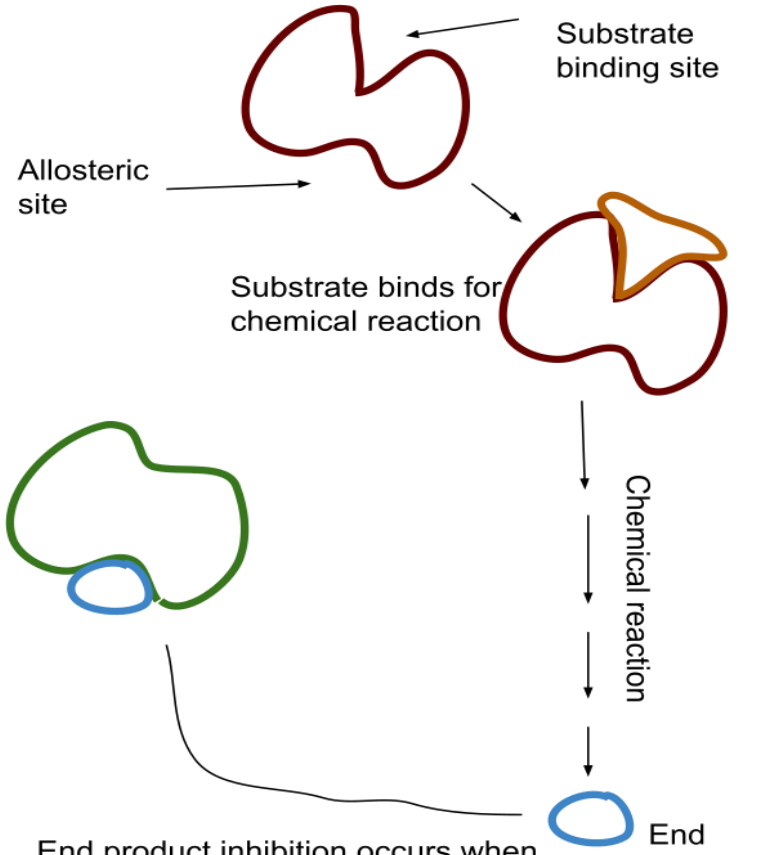


Both the the substrate and the inhibitor have the same shape causing both of them to compete for the substrate binding site

If the inhibitor binds to the enzyme the chemical reaction which would be carried out by the enzyme does not happen. The reaction is not carried out as no substrate can bind.

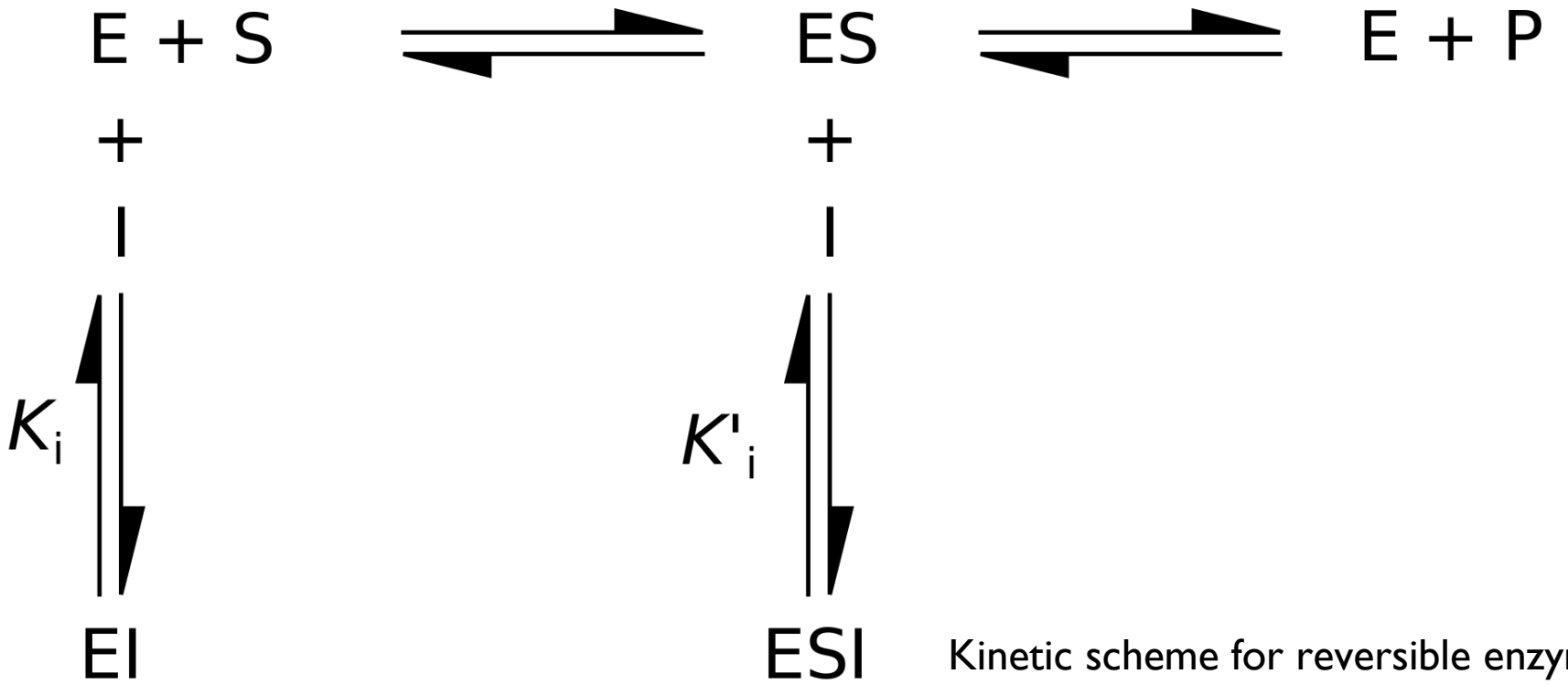
If the substrate binds to the substrate binding site, instead of the inhibitor, the chemical reaction is carried out and the end product is produced.

END-PRODUCT INHIBITION



End product inhibition occurs when too much of the end product is created causing there to be an excess. The extra product will bind to the allosteric site changing the shape of the substrate-binding site so the substrate can no longer bind stopping the metabolic pathway

Mixed-type inhibitors bind to both E and ES, but their affinities for these two forms of the enzyme are different (Ki ≠ Ki'). Thus, mixed-type inhibitors interfere with substrate binding (increase Km) and hamper catalysis in the ES complex (decrease Vmax).



Kinetic scheme for reversible enzyme inhibitors

Quantitative description of reversible inhibition

Reversible inhibition can be described quantitatively in terms of the inhibitor's binding to the enzyme and to the enzymesubstrate complex, and its effects on the kinetic constants of the enzyme. In the classic Michaelis-Menten scheme below, an enzyme (E) binds to its substrate (S) to form the enzyme–substrate complex ES. Upon catalysis, this complex breaks down to release product P and free enzyme. The inhibitor (I) can bind to either E or ES with the dissociation constants Ki or Ki', respectively.

Competitive inhibitors can bind to E, but not to ES. Competitive inhibition increases Km (i.e., the inhibitor interferes with substrate binding), but does not affect Vmax (the inhibitor does not hamper catalysis in ES because it cannot bind to ES). Uncompetitive inhibitors bind to ES. Uncompetitive inhibition decreases both Km' and 'Vmax. The inhibitor affects substrate binding by increasing the enzyme's affinity for the substrate (decreasing Km) as well as hampering catalysis (decreases Vmax). Non-competitive inhibitors have identical affinities for E and ES (Ki = Ki'). Non-competitive inhibition does not change Km (i.e., it does not affect substrate binding) but decreases Vmax (i.e., inhibitor binding hampers catalysis).

ALLOSTERIC ENZYMES AND THEIR KINETICS

Allosteric enzymes are enzymes that change their conformational ensemble upon binding of an effector (allosteric modulator) which results in an apparent change in binding affinity at a different ligand binding site. This "action at a distance" through binding of one ligand affecting the binding of another at a distinctly different site, is the essence of the allosteric concept. Allostery plays a crucial role in many fundamental biological processes, including but not limited to cell signaling and the regulation of metabolism. Allosteric enzymes need not be oligomers as previously thought, and in fact many systems have demonstrated allostery within single enzymes.

In biochemistry, allosteric regulation (or allosteric control) is the regulation of a protein by binding an effector molecule at a site other than the enzyme's active site

The site to which the effector binds is termed the allosteric site. Allosteric sites allow effectors to bind to the protein, often resulting in a conformational change involving protein dynamics. Effectors that enhance the protein's activity are referred to as allosteric activators, whereas those that decrease the protein's activity are called allosteric inhibitors.[citation needed]

Allosteric regulations are a natural example of control loops, such as feedback from downstream products or feedforward from upstream substrates. Long-range allostery is especially important in cell signaling. Allosteric regulation is also particularly important in the cell's ability to adjust enzyme activity.

The term allostery comes from the Greek allos (ἄλλος), "other," and stereos (στερεός), "solid (object)." This is in reference to the fact that the regulatory site of an allosteric protein is physically distinct from its active site. The protein catalyst (enzyme) may be part of a multi-subunit complex, and/or may transiently or permanently associate with a Cofactor (e.g. adenosine triphosphate). Catalysis of biochemical reactions is vital due to the very low reaction rates of the uncatalysed reactions. A key driver of protein evolution is the optimization of such catalytic activities via protein dynamics.

Whereas enzymes without coupled domains/subunits display normal Michaelis-Menten kinetics, most allosteric enzymes have multiple coupled domains/subunits and show cooperative binding. Generally speaking, such cooperativity results in allosteric enzymes displaying a sigmoidal dependence on the concentration of their substrates in positively cooperative systems. This allows most allosteric enzymes to greatly vary catalytic output in response to small changes in effector concentration. Effector molecules, which may be the substrate itself (homotropic effectors) or some other small molecule (heterotropic

effector), may cause the enzyme to become more active or less active by redistributing the ensemble between the higher affinity and lower affinity states. The binding sites for heterotropic effectors, called allosteric sites, are usually separate from the active site yet thermodynamically coupled

Kinetic properties

Hemoglobin, though not an enzyme, is the canonical example of an allosteric protein molecule - and one of the earliest to have its crystal structure solved (by Max Perutz). More recently, the E. coli enzyme aspartate carbamoyltransferase (ATCase) has become another good example of allosteric regulation.

The kinetic properties of allosteric enzymes are often explained in terms of a conformational change between a low-activity, low-affinity "tense" or T state and a high-activity, high-affinity "relaxed" or R state. These structurally distinct enzyme forms have been shown to exist in several known allosteric enzymes.

However the molecular basis for conversion between the two states is not well understood. Two main models have been proposed to describe this mechanism: the "concerted model" of Monod, Wyman, and Changeux, and the "sequential model" of Koshland, Nemethy, and Filmer

> The two principal models for the behavior of allosteric enzymes are the concerted model and the sequential model. They were proposed in 1965 and 1966, respectively, and both are currently used as a basis for interpreting experimental results. The concerted model has the advantage of comparative simplicity, and it describes the behavior of some enzyme systems very well.

> The sequential model sacrifices a certain amount of simplicity for a more realistic picture of the structure and behavior of proteins; it also deals very well with the behavior of some enzyme systems.

What is the concerted model for allosteric behavior?

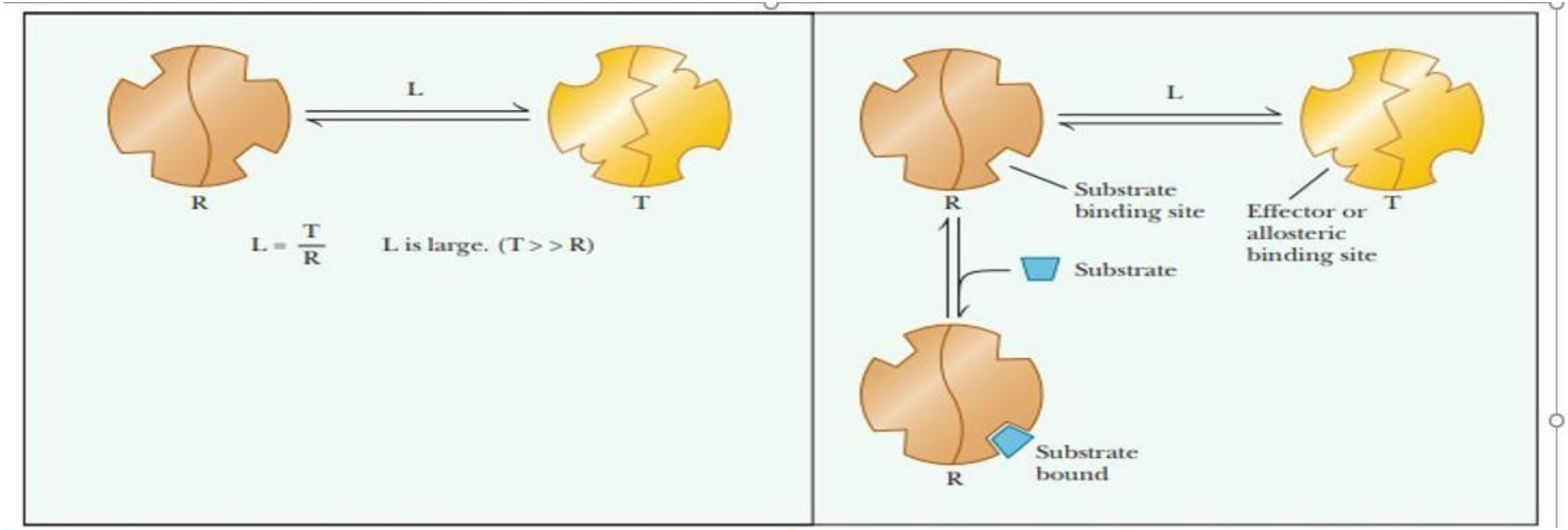
In 1965, Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux proposed the concerted model for the behavior of allosteric proteins in a paper that has become a classic in the biochemical literature. In this picture, the protein has two conformations, the active R (relaxed) conformation, which binds substrate tightly, and the inactive T (tight, also called taut)

conformation, which binds substrate less tightly. The distinguishing feature of this model is that the conformations of all subunits change simultaneously. Figure 1 shows a hypothetical protein with two subunits. Both subunits change conformation from the inactive T conformation to the active R conformation at the same time; that is, a concerted change of conformation occurs. The equilibrium ratio of the T/R forms is called L and is assumed to be high-that is, more enzyme is present in the unbound T form than in the unbound R form. The binding of substrate to either form can be described by the dissociation constant of the enzyme and substrate, K, with the affinity for substrate higher in the R form than in the T form. Thus, KR<<KT. The ratio of KR/KT is called c. Figure 2 shows a limiting

case in which KTis infinitely greater than KR(c = 0). In other words, substrate will not bindto the T form at all. The allosteric effect is explained by this model based on perturbing the equilibrium between the T and R forms. Although initially the amount of enzyme in the R form is small, when substrate binds to the R form, it removes free R form. This

causes the production of more R form to reestablish the equilibrium, which makes binding more substrate possible. This shifting of the equilibrium is responsible for the observed allosteric effects. The Monod–Wyman– Changeux model has been shown mathematically to explain the sigmoidal effects seen with allosteric enzymes. The shape of the curve will be based on the L and c values. As L increases (free T form more highly favored), the shape becomes more sigmoidal (Figure 3). As the value for c decreases (higher affinity between substrate and R form), the shape also becomes more sigmoidal.

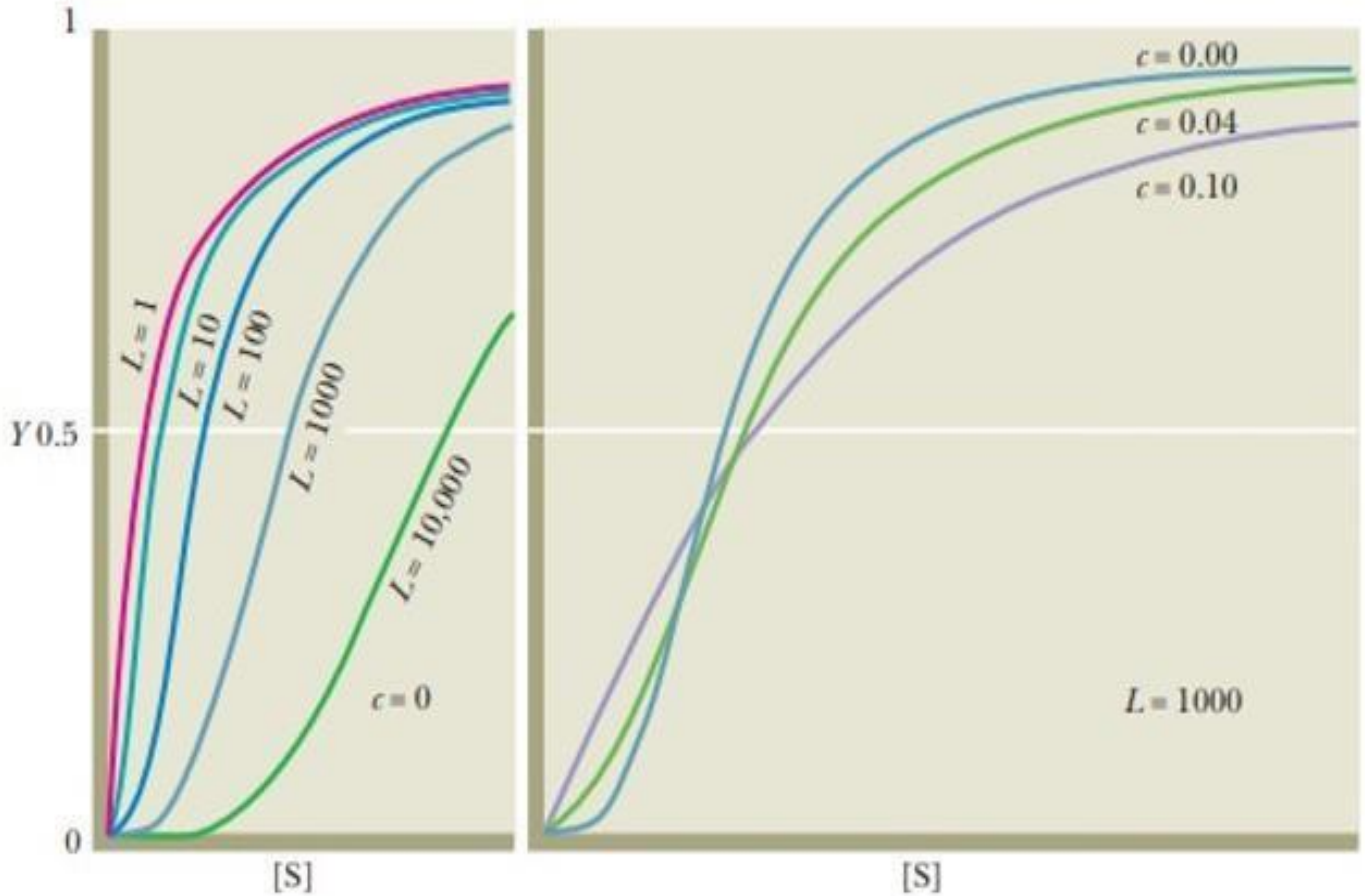
Monod Wyman-Changeux(MWC) model for allosteric transition is also called CONCENTRATED MODEL



A A dimeric protein can exist in either of two conformational states at equilibrium, the T (taut) form or the R (relaxed) form. L is the ratio of the T form to the R form. With most allosteric systems, L is large, so there is more enzyme present in the T form than in the R form.

B By Le Chatelier's principle, substrate binding shifts the equilibrium in favor of the relaxed state (R) by removing unbound R. The dissociation constant for the enzyme-substrate complex is K_R for the relaxed form and K_T for the taut form. $K_R < K_T$, so the substrate binds better to the relaxed form. The ratio of K_R / K_T is called c. This figure shows a limiting case in which the taut form does not bind substrate at all, in which case K_T is infinite and $c = 0$.

Monod Wyman-Changeux(MWC) model



A

As L (the ratio of the T/R form) increases, the shape becomes more sigmoidal.

B

The level of cooperativity is also based on the affinity of the substrates for the T or R form. When K_T is infinite (zero affinity), cooperativity is high, as shown in the blue line, where $c = 0$ ($c = K_R/K_T$). As c increases, the difference in binding between the T and R forms decreases, and the lines become less sigmoidal.

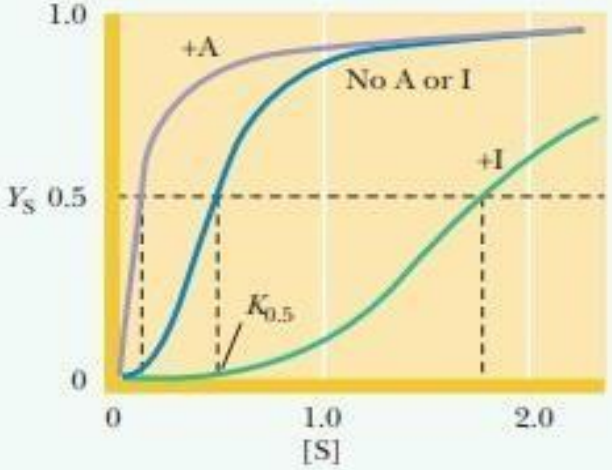
In the concerted model, the effects of inhibitors and activators can also be considered in terms of shifting the equilibrium between the T and R forms of the enzyme. The binding of inhibitors to allosteric enzymes is cooperative; allo-steric inhibitors bind to and stabilize the T form of the enzyme. The binding of activators to allosteric enzymes is also cooperative; allosteric activators bind to and stabilize the R form of the enzyme. When an activator, A, is present, the cooperative binding of A shifts the equilibrium between the T and R forms, with the R form favored (Figure). As a result, there is less need for substrate, S, to shift the equilibrium in favor of the R form, and less cooperativity in the binding of S is seen.

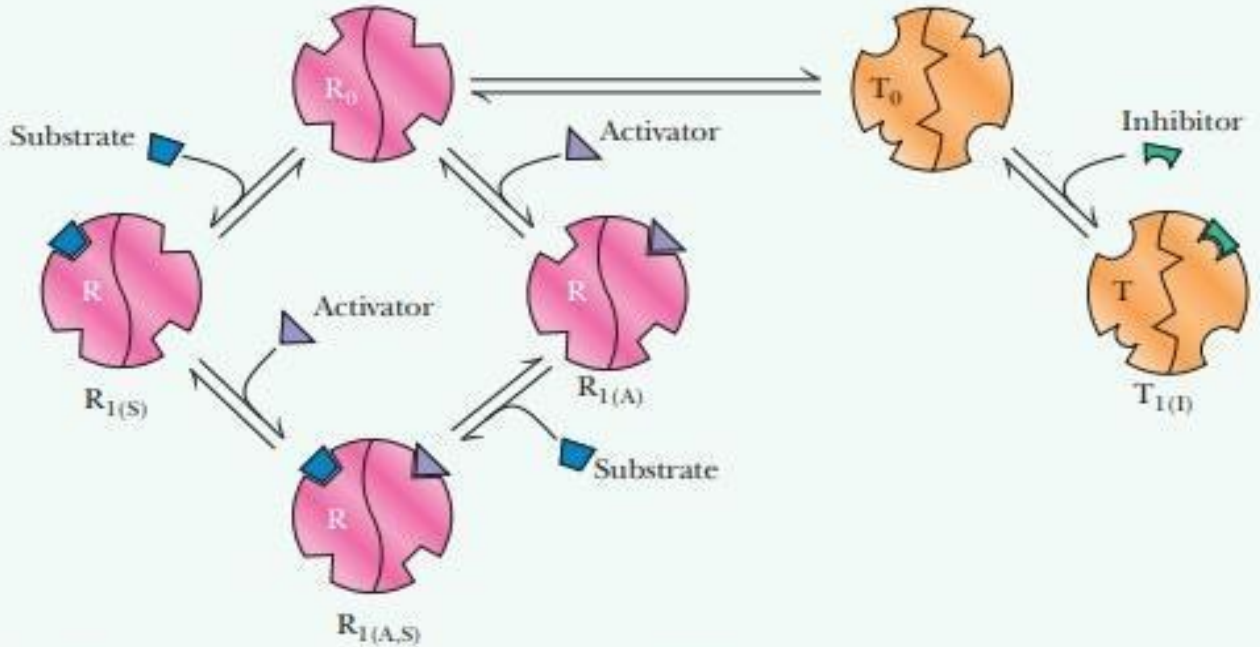
A dimeric protein that can exist in either of two states: R_0 or T_0.
This protein can bind three ligands:

1) Substrate (S): A positive homotropic effector that binds only to R at site S

2) Activator (A): A positive heterotropic effector that binds only to R at site F

3) Inhibitor (I): A negative heterotropic effector that binds only to T at site F





Effects of A:
$A + R_0 \rightarrow R_{1(A)}$
Increase in number of R-conformers shifts $R_0 \rightleftharpoons T_0$ so that $T_0 \rightarrow R_0$

(1) More binding sites for S made available.

(2) Decrease in cooperativity of substrate saturation curve. Effector A lowers the apparent value of *L*.

Effects of I:
$I + T_0 \rightarrow T_{1(I)}$
Increase in number of T-conformers (decrease in R_0 as $R_0 \rightarrow T_0$ to restore equilibrium)

Thus, I inhibits association of S and A with R by lowering R_0 level. I increases cooperativity of substrate saturation curve. I raises the apparent value of *L*.

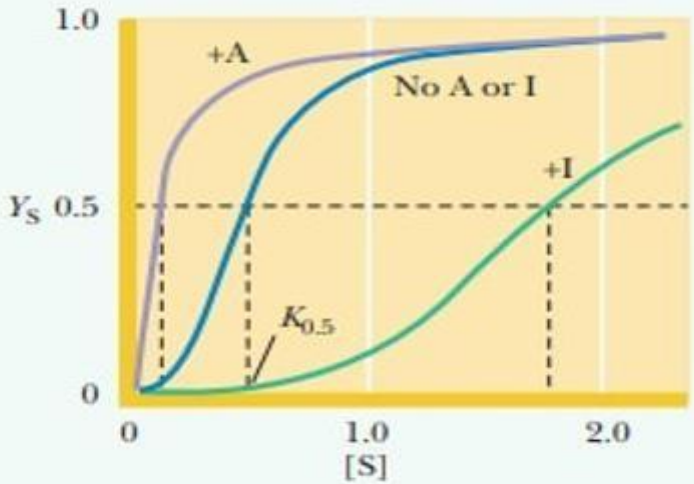
■ **ACTIVE FIGURE 7.6** **Effects of binding activators and inhibitors with the concerted model.** An activator is a molecule that stabilizes the R form. An inhibitor stabilizes the T form.

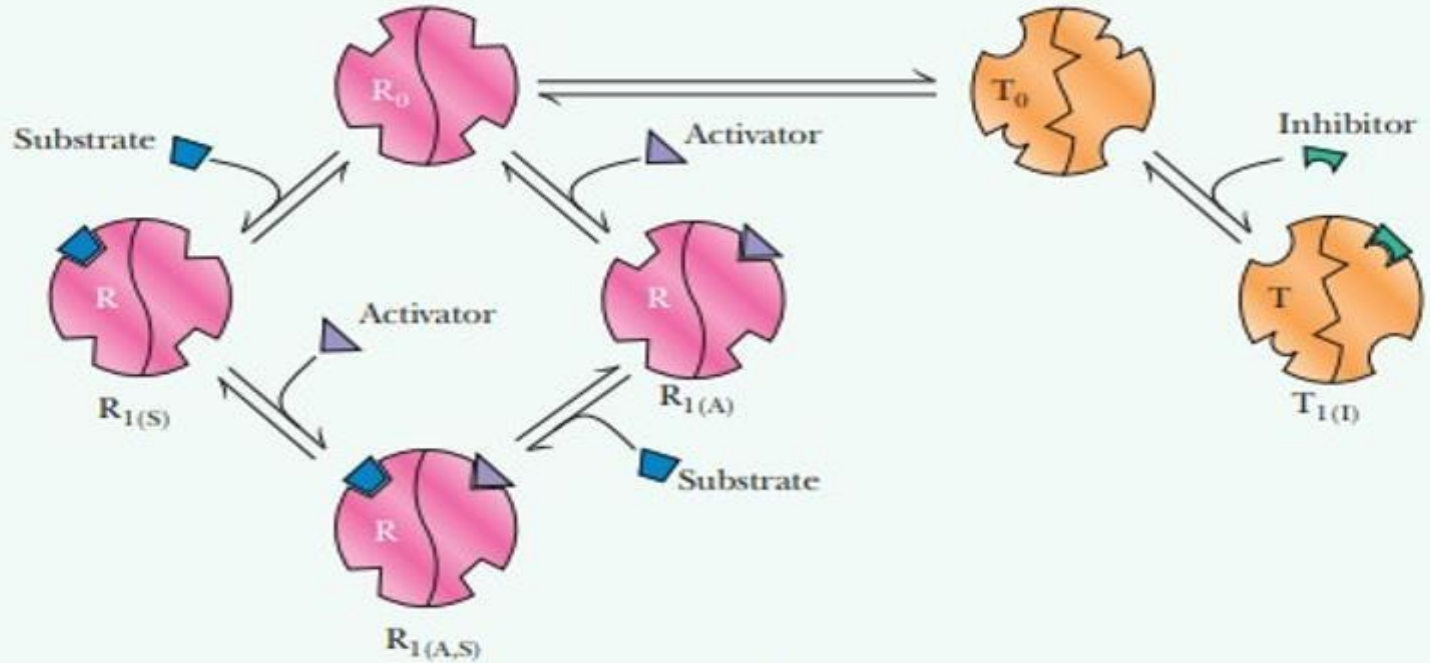
A dimeric protein that can exist in either of two states: R_0 or T_0.
This protein can bind three ligands:

1) Substrate (S): A positive homotropic effector that binds only to R at site S

2) Activator (A): A positive heterotropic effector that binds only to R at site F

3) Inhibitor (I): A negative heterotropic effector that binds only to T at site F





Effects of A:
$A + R_0 \rightarrow R_{1(A)}$
Increase in number of R-conformers shifts $R_0 \rightleftharpoons T_0$ so that $T_0 \rightarrow R_0$

(1) More binding sites for S made available.

(2) Decrease in cooperativity of substrate saturation curve. Effector A lowers the apparent value of L.

Effects of I:
$I + T_0 \rightarrow T_{1(I)}$
Increase in number of T-conformers (decrease in R_0 as $R_0 \rightarrow T_0$ to restore equilibrium)

Thus, I inhibits association of S and A with R by lowering R_0 level. I increases cooperativity of substrate saturation curve. I raises the apparent value of L.

Effects of binding activators and inhibitors with the concentrated model

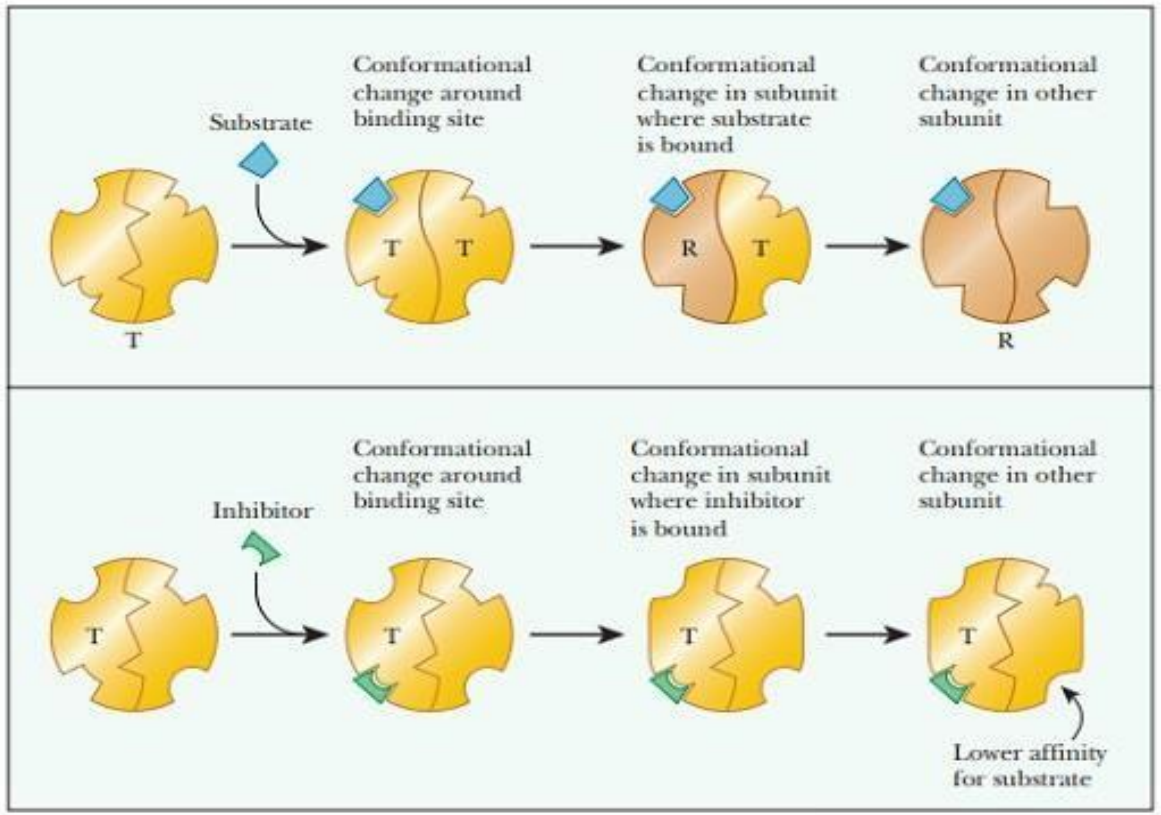
When an inhibitor, I, is present, the cooperative binding of I also shifts the equilibrium between the T and R forms, but this time the T form is favored (Figure 7.6). More substrate is needed to shift the T-to-R equilibrium in favor of the R form. A greater degree of cooperativity is seen in the binding of S.

What is the sequential model for allosteric behavior?

The name Daniel Koshland is associated with the direct sequential model of allosteric behavior. The distinguishing feature of this model is that the binding of substrate induces the conformational change from the T form to the R form-the type of behavior postulated by the induced-fit theory of substrate binding. A conformational change from T to R in one subunit makes the same conformational change easier in another subunit, and this is the form in which cooperative binding is expressed in this model (Figure).

In the sequential model, the binding of activators and inhibitors also takes place by the induced-fit mechanism. The conformational change that begins with binding of inhibitor or activator to one subunit affects the conformations of other

subunits. The net result is to favor the R state when activator is present and to favor the T form when inhibitor, I, is present (Figure).



A Sequential model of cooperative binding of substrate S to an allosteric enzyme. Binding substrate to one subunit induces the other subunit to adopt the R state, which has a higher affinity for substrate.

B Sequential model of cooperative binding of inhibitor I to an allosteric enzyme. Binding inhibitor to one subunit induces a change in the other subunit to a form that has a lower affinity for substrate.

FIGURE 7.7

Binding I to one subunit causes a conformational change such that the T form is even less likely to bind substrate than before. This conformational change is passed along to other subunits, making them also more likely to bind inhibitor and less likely to bind substrate. This is an example of cooperative behavior that leads to more inhibition of the enzyme. Likewise, binding an activator causes a conformational change that favors substrate binding, and this effect is passed from one subunit to another.

The sequential model for binding effectors of all types, including substrates, to allosteric enzymes has a unique feature not seen in the concerted model. The conformational changes thus induced can make the enzyme less likely to bind more molecules of the same type. This phenomenon, called negativecooperativity, has been observed in a few enzymes

One is tyrosyl tRNA synthe-tase, which plays a role in protein synthesis. In the reaction catalyzed by this enzyme, the amino acid tyrosine forms a covalent bond to a molecule of trans-fer RNA (tRNA). In subsequent steps, the tyrosine is

passed along to its place in the sequence of the growing protein. The tyrosyl tRNA synthetase consists of two subunits. Binding of the first molecule of substrate to one of the subunits inhibits binding of a second molecule to the other subunit.

The sequential model has successfully accounted for the negative coopera-tivity observed in the behavior of tyrosyl tRNA synthetase. The concerted model makes no provision for negative cooperativity.

Summary

The two principal models for allosteric enzyme behavior are called the concerted model and the sequential model.

In the concerted model, the enzyme is thought of as being in a taut form, T, or a relaxed form, R. All subunits are found in one or the other, and an equilibrium exists between the T and R forms.

Substrate binds more easily to the R form than to the T form, inhibitors stabilize the T form, and activators stabilize the R form.

In the sequential model, subunits of the enzyme can change sequentially from the T form to the R form and back again.

Binding of one molecule of substrate to one subunit stimulates the transi-tion of the subunit to the R form, which then stimulates another subunit to change to the R form.

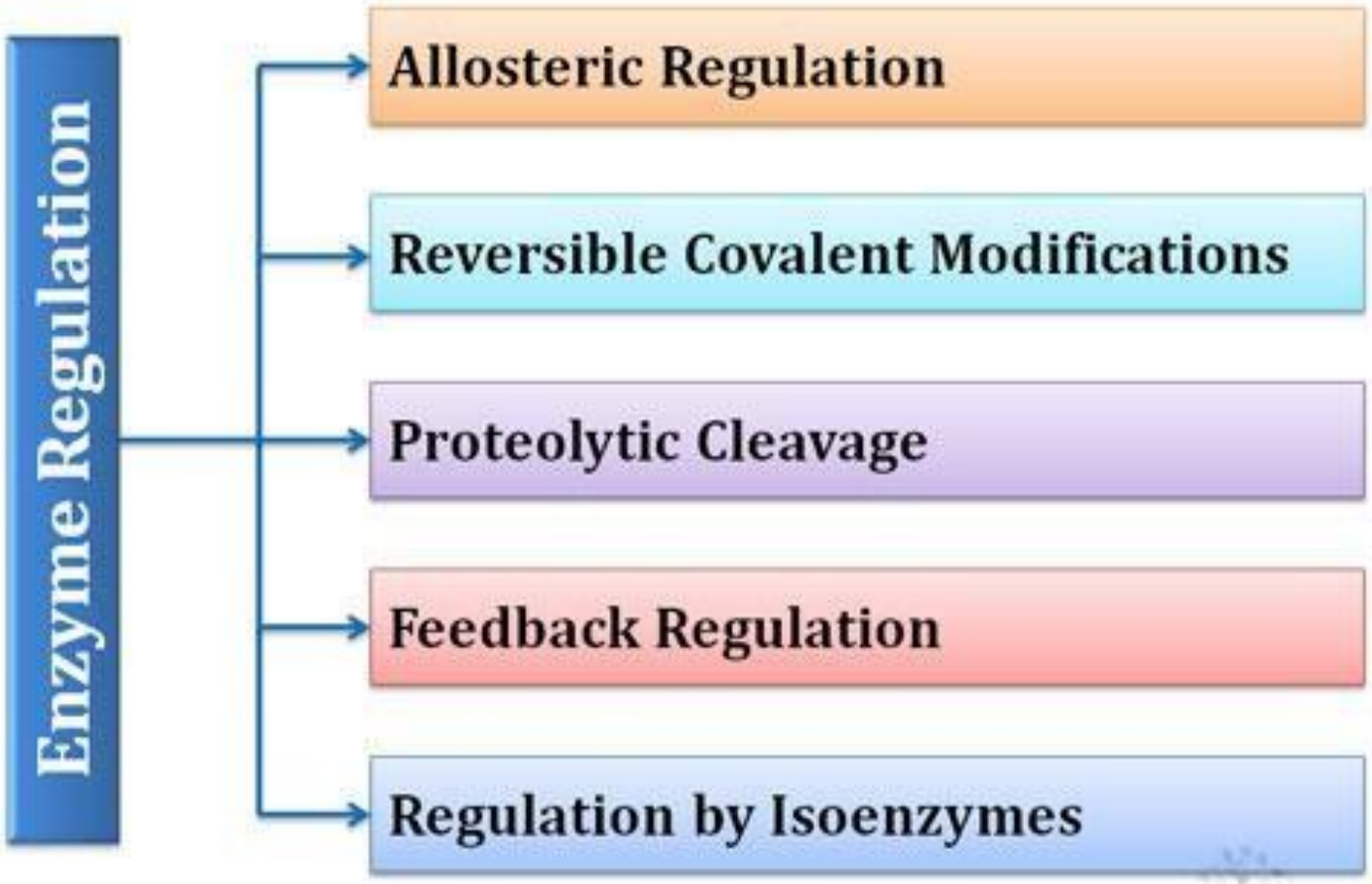
Binding of inhibitor to one subunit induces a change in the other sub-units to a form with lower affinity for the substrate.
Binding of an activator to one subunit induces a shift in the other subunits to a form that has a high affinity for substrate.

Properties of Allosteric enzymes

1. Catalyze essentially irreversible reactions; are rate limiting
2. Generally contain more than one polypeptide chain
3. Do not follow Michaelis-Menten Kinetics
4. Are regulated by allosteric activators or inhibitors
5. Can be up-regulated by allosteric activators at constant [S]
6. Can be down regulated by allosteric inhibitors at constant [S]
7. Activators and Inhibitors need not have any structural resemblance to substrate structure

REGULATION OF ENZYME ACTION

Enzymes can be regulated by other molecules that either increase or reduce their activity. Molecules that increase the activity of an enzyme are called activators, while molecules that decrease the activity of an enzyme are called inhibitors.



Methods of Enzyme Regulation

Regulation of enzyme activity is important to coordinate the different metabolic processes.
It is also important for homeostasis i.e. to maintain the internal environment of the organism constant.
Regulation of enzyme activity can be achieved by two general mechanisms:

1. **Control of enzyme quantity**
   Enzyme quantity is affected by:
   A- Altering the rate of enzyme synthesis and degradation,
   B- Induction
   C- Repression
2. **Altering the catalytic efficiency of the enzyme by**
   Catalytic efficiency of enzymes is affected by:
   A- Allosteric regulation
   B- Feedback inhibition
   C- Proenzyme (zymogen)
   D- Covalent modification
   E- Protein - Protein interaction

1- Control of enzyme quantity

A- Control of the rates of enzyme synthesis and degradation.

As enzymes are protein in nature, they are synthesized from amino acids under gene control and degraded again to amino acids after doing its work.
Enzyme quantity depends on the rate of enzyme synthesis and the rate of its degradation.

- Increased enzyme quantity may be due to an increase in the rate of synthesis, a decrease in the rate of degradation or both.
- Decreased enzyme quantity may be due to a decrease in the rate of synthesis, an increase in the rate of degradation or both.

B- Induction

- Induction means an increase in the rate of enzyme synthesis by substances called inducers
- According to the response to inducers, enzymes are classified into:
  i- Constitutive enzymes, the concentration of these enzymes does not depend on inducers.
  ii- Inducible enzymes, the concentration of these enzymes depends on the presence of inducers
- For example, induction of lactase enzyme in bacteria grown on glucose media.

C- Repression

- Repression means a decrease in the rate of enzyme synthesis by substances called repressors.
- Repressors are low molecular weight substances that decrease the rate of enzyme synthesis at the level of gene expression.
- Repressors are usually end products of biosynthetic reaction, so repression is sometimes called feedback regulation.
- For example, dietary cholesterol decreases the rate of synthesis of HMG CoA reductase (β-hydroxy β- methyl glutaryl CoA reductase), which is a key enzyme in cholesterol biosynthesis.

D- Derepression

Following removal of the repressor or its exhaustion, enzyme synthesis retains its normal rate.

E- Concentration of substrates, coenzymes and metal ion activator

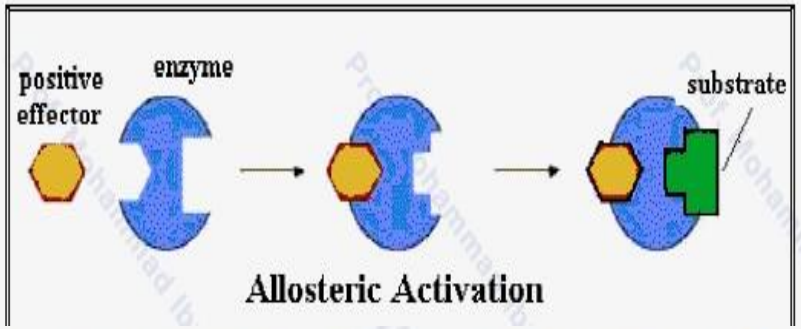
The susceptibility of the enzyme to degradation depends on its conformation. Presence of substrate, coenzyme or metal ion activator causes changes in the enzyme conformation decreasing its rate of degradation.

The susceptibility of the enzyme to degradation depends on its conformation. Presence of substrate, coenzyme or metal ion activator causes changes in the enzyme conformation decreasing its rate of degradation.

2- Control of catalytic efficiency of enzymes

A- Allosteric Regulation

Allosteric enzyme is formed of more than one protein subunit. It has two sites; a catalytic site for substrate binding and another site (allosteric site), that is the regulatory site, to which an effector binds.
Allosteric means another site
If binding of the effector to the enzyme increases it activity, it is called positive effector or allosteric activator e.g. ADP is allosteric activator for phosphofructokinase enzyme.



If binding of the effector to the enzyme causes a decrease in its activity, it is called negative effector or allosteric inhibitor e.g.

- ATP and citrate are allosteric inhibitors for phosphofructokinase enzyme.
- Glucose-6-phosphate is allosteric inhibitor for hexokinase enzyme.

Regulation of Metabolic Pathways A - How is Enzyme Activity Regulated?

Exquisite mechanisms have evolved that control the flux of metabolites through metabolic pathways to insure that the output of the pathways meets biological demand and that energy in the form of ATP is not wasted by having opposing pathways run concomitantly in the same

cell.

Enzymes can be regulated by changing the activity of a preexisting enzyme or changing the amount of an enzyme.

A. Changing the activity of a pre-existing enzyme: The quickest way to modulate the activity of an enzyme is to alter the activity of an enzyme that already exists in the cell. The list below, illustrated in the following figure, gives common ways to regulate enzyme activity Substrate availability: Substrates (reactants) bind to enzymes with a characteristic affinity (characterized by a dissociation constant) and a kinetic parameter called Km (units of molarity). If the actual concentration of a substrate in a cell is much less than the Km, the activity of the enzyme is very low. If the substrate concentration is much greater than Km, the enzyme active site is saturated with substrate and the enzyme is maximally active.

Product inhibition: A product of an enzyme-catalyzed reaction often resembles a starting reactant, so it should be clear that the product should also bind to the activity site, albeit probably with lower affinity. Under conditions in which the product of a reaction is present in high concentration, it would be energetically advantageous to the cell if no more product was synthesized. Product inhibition is hence commonly observed. Likewise it be energetically advantageous to a cell if the end product of an entire pathway could likewise bind to the initial enzyme in the pathways and inhibit it, allowing the whole pathway to be inhibited. This type of feedback inhibition is commonly oberved

Allosteric regulation: As many pathways are interconnected, it would be optimal if the molecules of one pathway affected the activity of enzymes in another interconnected pathway, even if the molecules in the first pathway are

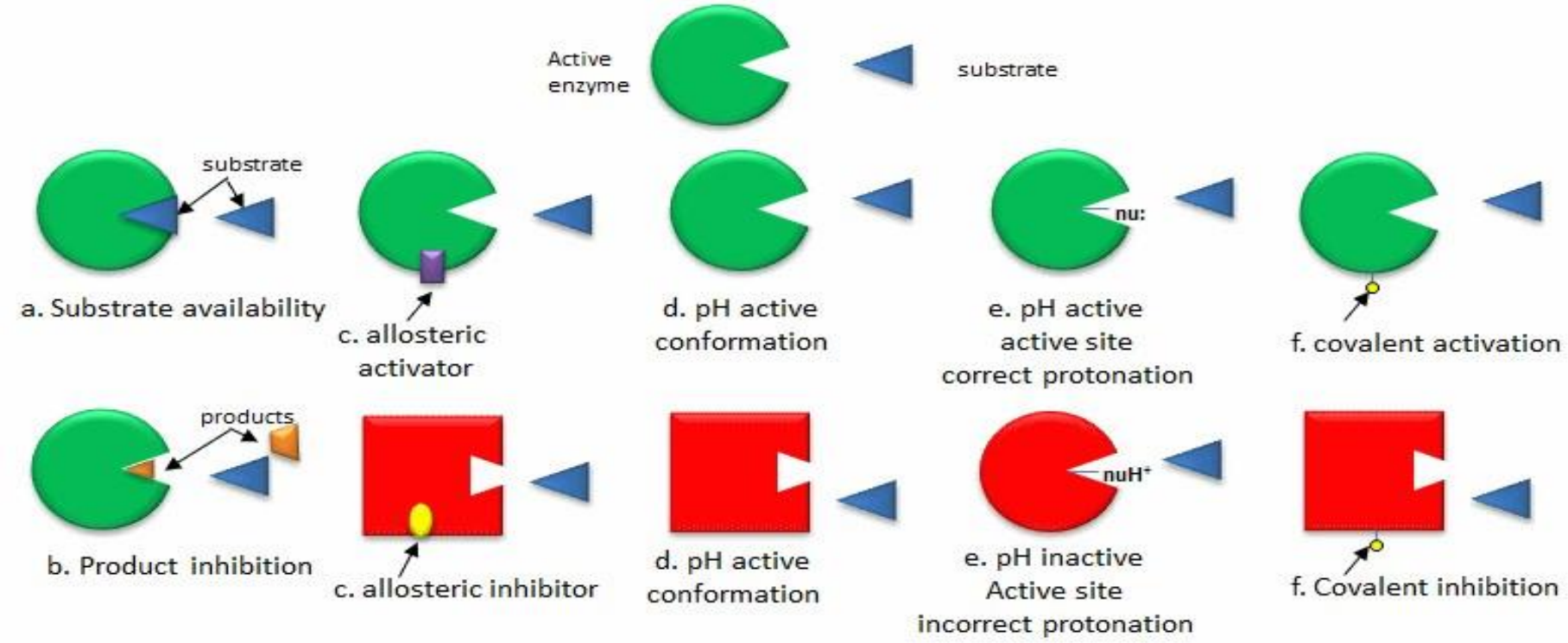
structurally dissimilar to reactants or products in a second pathway. Molecules that bind to sites on target enzymes other than the active site (allosteric sites) can regulate the activity of the target enzyme. These molecules can be structurally dissimilar to those that bind at the active site. They do so my conformational changes which can either activate or inhibit the target enzyme's activity.

pH and enzyme conformation: Changes in pH which can accompany metabolic process such as respiration (aerobic glycolysis for example) can alter the conformation of an enzyme and hence enzyme activity. The initial changes are covalent (change in protonation state of the protein) which can lead to an alteration in the delicate balance of forces that affect protein structure.

pH and active site protonation state: Changes in pH can affect the protonation state of key amino acid side chains in the active site of proteins without affecting the local or global conformation of the protein. Catalysis may be affected if the mechanism of catalysis involves an active site nucleophile (for example), that must be deprotonated for activity.

Covalent modification: Many if not most proteins are subjected to post-translational modifications which can affect enzyme activity through local or global shape changes, by promoting or inhibiting binding interaction of substrates

and allosteric regulators, and even by changing the location of the protein within the cell. Proteins may be phosphorylated, acetylated, methylated, sulfated, glycosylated, amidated, hydroxylated, prenylated, myristolated, often in a reversible fashion. Some of these modifications are reversible. Regulation by phosphorylation through the action of kinases, and dephosphorylation by phosphates is extremely common. Control of phosphorylation state is mediated through signal transduction process starting at the cell membrane, leading to the activation or inhibition of protein kinases and phosphatases within the cell.



Extracellular regulated kinase 2 (ERK2), also known as mitogen activate protein kinase 2 (MAPK2) is a protein the plays a vital role in cell signaling across the cell membrane. Phosphoryation of ERK2 on Threonine 183 (Thr153) and Tyrosine 185 (Tyr185) leads to a structural change in the protein and the regulation of its activity.

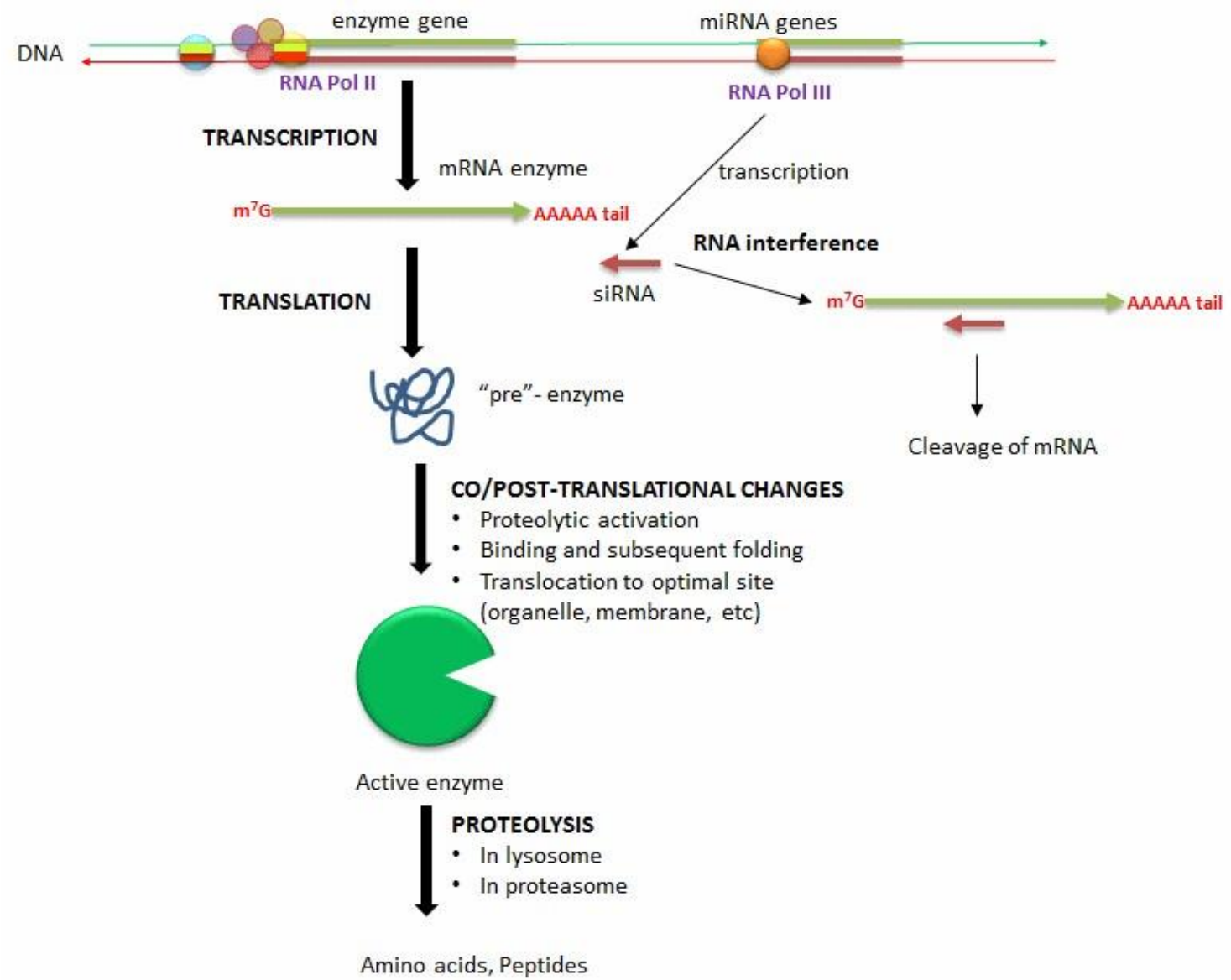
Fig-Structural Comparison of phosphorylated and dephosphorylated enzyme

B. Changing the amount of an enzyme: **Another and less immediate but longer duration method to modulate the activity of an enzyme is to alter the activity of an enzyme that already exists in the cell. The list below, illustrated in the following figure, shows way in which enzyme concentration is regulated.**

Alternation in transcription of enzyme's gene: Extracellular signal (hormones, neurotransmitters, etc) can lead to signal transductions responses and ultimate activation or inhibition of the transcription of the gene for a protein enzyme. These changes result from recruitment of transcription factors (proteins) to DNA sequences that regulate transcription of the enzyme gene.

Degradation of messenger RNA for the enzyme: The levels of messenger RNA for a protein will directly determin the amount of that protein synthesized. Small inhibitor RNAs, derived from microRNA molecules transcribed from cellular DNA, can bind to specific sequences in the mRNA of a target enzyme. The resulting double-stranded RNA complex recruits an enzyme (Dicer) that cleaves the complex with the effect of decreasing translation of the protein enzyme from its mRNA.

Co/Post translational changes: **Once a protein enzymes is translated from its mRNA, it can undergo a changes to affect enzyme levels. Some proteins are synthesized in a "pre"form which must be cleaved in a targeted and limited fashion by proteases to active the protein enzyme. Some proteins are not fully folded and must bind to other factors in the cell to adopted a catalytically active form. Finally, fully active protein can be fully proteolyzed by the proteasome, a complex within cells, or in lysosomes, which are organelles within cells containing proteolytic enzymes.**


DNA
enzyme gene
miRNA genes
RNA Pol II
RNA Pol III
TRANSCRIPTION
mRNA enzyme
transcription
m7G
AAAAA tail
RNA interference
siRNA
TRANSLATION
m7G
AAAAA tail
"pre"- enzyme
Cleavage of mRNA
CO/POST-TRANSLATIONAL CHANGES
• Proteolytic activation
• Binding and subsequent folding
• Translocation to optimal site (organelle, membrane, etc)
Active enzyme
PROTEOLYSIS
• In lysosome
• In proteasome
Amino acids, Peptides