

Enzyme

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Life is possible by virtue of the coordination of various chemical reactions that constantly take place in living organisms, such as the digestion of food, absorption of necessary molecules, and production of energy. This process involves a sequence of reactions that take place within your body under very mild conditions. All of this occurs with the help of certain biocatalysts known as **enzymes**.

Enzyme activity depends on both reaction chemistry and the conditions of the assay. A kinetic parameter should be interpreted within its model.

The term *enzyme* was first coined by Wilhelm Kuhne in 1877, which roughly means "in yeast" in Greek (referring to the fermentation of sucrose to alcohol by zymase found in yeasts). In 1926, Sumner first crystallized the enzyme **urease** in pure form from jack beans (*Canavalia ensiformis*). For this work, he was awarded the Nobel Prize in Chemistry in 1946 along with Stanley and Northrop.

Almost all enzymes have been found to be globular proteins in nature, although some RNA molecules also catalyze reactions. Catalytic RNAs are called **ribozymes**; RNase P is one example. These catalytic RNA molecules, known as **ribozymes**, were discovered by Thomas Cech and Sidney Altman, for which they were awarded the Nobel Prize in Chemistry (1989).

Structure of an Enzyme

Like any other protein, an enzyme possesses the primary, secondary, and tertiary structures. When you observe the tertiary structure of an enzyme, you'll notice that the backbone of the protein chain folds upon itself, criss-crosses itself, and leads to the formation of many crevices or pockets.

One such pocket is the **active site** into which the substrate fits during a catalysis reaction. With the help of their active site, enzymes catalyse reactions at a high rate. Unlike inorganic catalysts that you may have read about in chemistry, enzymes can't usually work efficiently at high temperatures; being proteins, they are denatured at high temperatures (at temperatures that depend on the particular enzyme).

However, special enzymes isolated from organisms that normally live under extremely high temperatures, such as hot vents and sulphur springs, are stable and retain their catalytic power even

at high temperatures (around 80° to 90°C). Thermal stability is an important virtue of such enzymes isolated from thermophilic organisms, and they are extensively used in biotechnological processes.

Most enzymes in the human body work best at around 37°C and a pH close to 7.4. If you're studying for exams, remember this: temperature and pH don't "kill" an enzyme directly. They disrupt the hydrogen bonds and ionic interactions that hold the enzyme's 3D shape together, which is what destroys catalytic function.

Properties of Enzymes

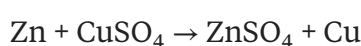
Enzymes have certain distinct properties that you should know about:

1. **Most enzymes are chemically proteins, which are one of the key biomolecules found in living organisms.** They may possess additional inorganic or organic groups known as **co-factors** that are necessary for their biological activity (discussed later).
2. Like inorganic catalysts, enzymes don't actually start a **chemical reaction** but increase the rate of an ongoing reaction by *decreasing the magnitude of the activation energy*. They don't change the equilibrium but help the equilibrium state get attained faster.
3. The number of substrate molecules changed per minute by an enzyme molecule is known as the **turnover number**. The higher the turnover number, the higher the enzyme's efficiency.
4. Being catalysts, enzymes aren't transformed or used up in the chemical reaction. They are released unchanged at the end of the reaction.
5. Unlike inorganic catalysts, enzymes are **highly specific in their action for a particular reaction and for a particular substrate**. For example, the enzyme maltase acts only on the sugar maltose, not on sucrose or lactose.

How Do Enzymes Catalyze Biochemical Reactions?

To understand how enzymes work, you need to first take a closer look at chemical reactions in general. Chemical compounds can potentially undergo two types of changes: physical and chemical. A **physical change** simply refers to a change in physical shape without involving any breaking of chemical bonds. This also includes a change in state of matter, such as ice melting or water turning into vapour.

On the other hand, when bonds are broken and new bonds are formed during the transformation, a chemical reaction is said to take place. The hydrolysis of starch into glucose is an example of an organic chemical reaction. Given below is an example of an inorganic chemical reaction.



The **rate** of a physical or chemical process refers to the **amount of product formed per unit of time**. It can be expressed in the following manner:

$$\text{Rate} = \delta P / \delta t$$

If the direction happens to be specified, then the rate may also be known as the **velocity**. Several factors, including the temperature, influence the rates of physical and chemical processes. A general rule of thumb is that the rate gets doubled or halved for every change of 10°C in either direction.

Catalyzed reactions have been observed to proceed at much higher rates than the ones that aren't. When you study enzyme-catalyzed reactions, you'll find their rate is much higher than the same reaction without a catalyst.

Consider a relatively simple reaction: carbon dioxide getting dissolved in water to produce carbonic acid (H_2CO_3). In the absence of an enzyme, this reaction proceeds very slowly with only about 200 molecules of carbonic acid being formed every hour. However, in the presence of an enzyme named **carbonic anhydrase**, the reaction speeds up nearly 10 million times with about 600,000 molecules being formed every second. That's a staggering difference, and it shows you just how powerful enzymes are.

In your body, there are thousands of different types of enzymes. Each of these enzymes catalyzes a unique biochemical reaction. A multistep chemical reaction where each of the steps is catalyzed by the same enzyme complex or different enzymes is known as a **metabolic pathway**.

The oxidation of glucose to pyruvic acid through ten different enzyme-catalyzed metabolic reactions is a good example of a metabolic pathway. With one or two additional reactions, this same pathway can give rise to a range of diverse metabolic end products. For example, lactic acid is formed in your skeletal muscles under anaerobic conditions while ethyl alcohol is produced in yeast **cells** during fermentation. Hence, different products can form in different conditions.

Mechanism of Enzyme Action

Now that you understand the concept of an "active site," let's look at how the catalytic process actually works. The chemical or metabolic conversion refers to a reaction, whereas the chemical being converted into a product is known as the **substrate**. Consider a hypothetical enzyme converting a substrate (S) into a product (P), in a reaction that may be depicted as:



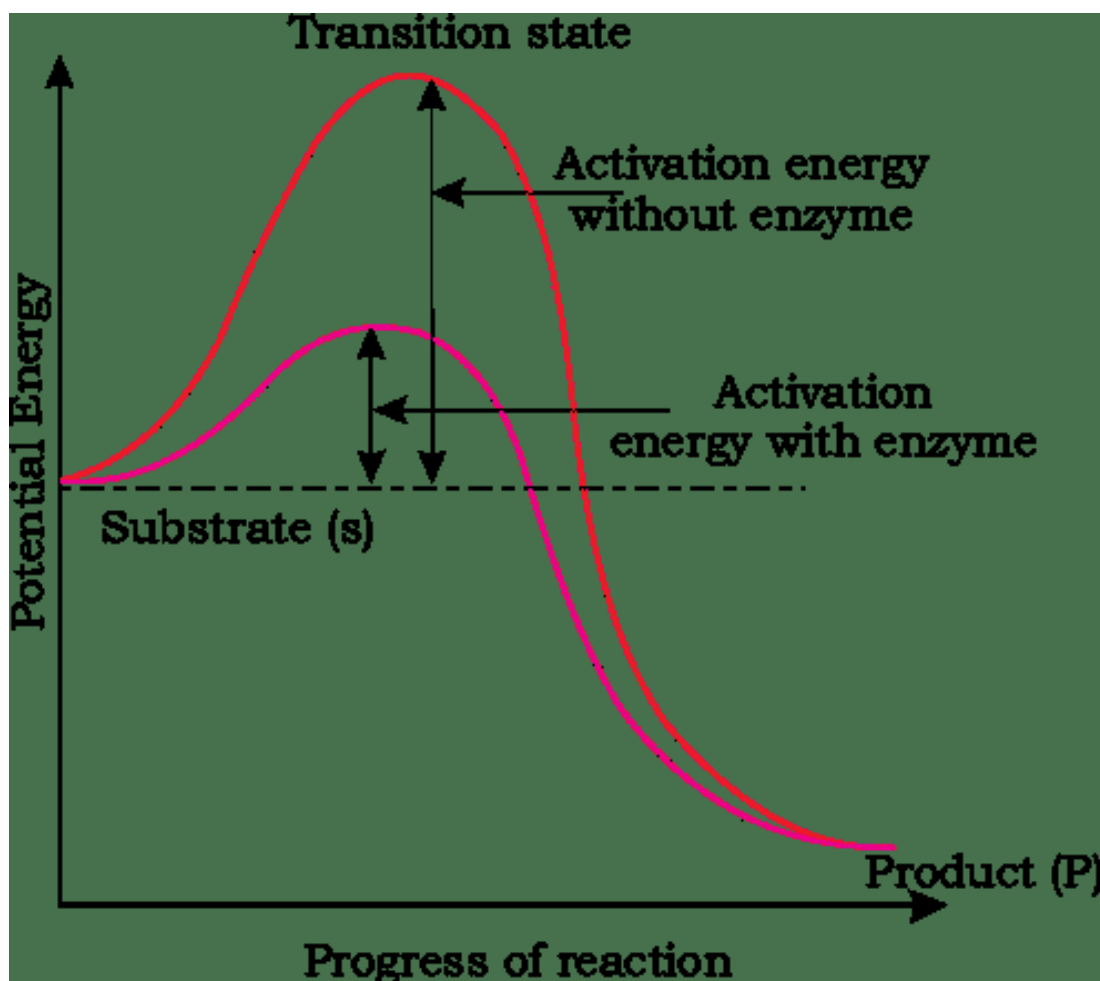
The substrate S needs to bind to the enzyme at its active site within a particular cleft or pocket. Because the substrate has to diffuse towards the active site, there is an inevitable formation of an **ES complex**. Here, "E" stands for the enzyme.

This complex formation is only a transient phenomenon. When the substrate is bound to the active

site of the enzyme, a new structure of the substrate known as the **transition state** is formed. Soon afterward, the structure of the substrate gets transformed into that of the product and is released from the active site.

The pathway of this transformation must inevitably go through this transition state structure at some point. There could be many more intermediate "altered structural states" between the stable substrate and the product, all of which are unstable.

Stability depends greatly upon the energy status of the molecule or the structure you're dealing with. You can understand this concept better using the graph shown below:



Catalyzed and uncatalyzed reaction-energy paths with different activation barriers

Here, the y-axis represents the **potential energy content** whereas the x-axis represents the **progression of the reaction**. Take note of two things here: firstly, the energy level difference between S and P. If P is at a lower level than S, the reaction is an **exothermic reaction**. In this case, you don't need to supply energy (in the form of heat) in order to get the product.

However, in the case of both exothermic and endothermic reactions, the substrate S has to go through a much higher energy state or transition state. The difference in the average energy content of S from that of this transition state is known as the **activation energy**.

Enzymes work by bringing down this energy barrier and making the transition of S to P much

easier. The ES complex is short-lived and soon dissociates into its product(s) P and the unaltered enzyme. The formation of the ES complex is absolutely essential for catalysis.

Models for the Mode of Enzyme Action

Scientists have put forward three different models to explain the mode of enzyme action. Let's walk through each one.

Lock and key hypothesis

It was proposed by **Emil Fischer** in 1894. According to this model, the enzyme has a rigid structure or conformation. The substrate fits into the active site of the enzyme just like a key only fits into the proper lock. The active site contains special groups having -NH_2 , -COOH , and -SH moieties to establish contact with substrate molecules.

After the substrate molecules come in contact with the active site of the enzyme, they undergo chemical changes and form products. Because the product no longer fits into the active site, it is released to the surrounding medium and leaves the active site free to receive more substrate molecules. Essentially, the hypothesis states that the active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind.

This model demonstrates how a small concentration of an enzyme can act upon a large amount of the substrate. It explains why the enzyme remains unaffected at the end of the reaction. It also explains how competitive inhibitors possessing a structure similar to the substrate inhibit the enzyme.

However, the lock and key model doesn't adequately explain the flexible nature of enzymes. It fails to explain many features of enzymatic reactions such as the effect of allosteric modulators.

Induced fit hypothesis

In 1958, **Koshland** proposed a more acceptable and realistic model for enzyme-substrate complex formation. According to this hypothesis, the active site of the enzyme doesn't initially exist in a shape complementary to the substrate. Rather, it is induced to assume the said shape when the substrate is bound to the enzyme.

Think of it this way: the active site is induced to assume a complementary shape much like a hand induces a change in the shape of a glove. The model asserts that enzymes or their active sites are flexible in nature. The active site of the enzyme possesses two groups:

1. **A buttressing group** for supporting the substrate.
2. **A catalytic group** for catalysing the reaction.

When the substrate comes in contact with the buttressing group, the active site becomes modified to bring the catalytic group opposite to the substrate bonds, which are then broken.

The induced fit model is supported by strong experimental evidence from X-ray diffraction studies. It also explains the action of allosteric modulators and competitive inhibitors on enzymes.

Substrate strain theory

In the substrate strain theory, the substrate is strained due to the induced conformation change in the enzyme. It is also possible that when a substrate binds to the preformed active site, the enzyme induces a strain on the substrate that leads to the formation of the product.

Some scientists feel that a combination of the induced fit model with the substrate strain is operative in the mechanism of enzymatic action.

Catalytic Cycle of Enzyme Action

You can describe the catalytic cycle of enzyme action in the following steps:

1. First, the substrate binds to and fits into the active site of the enzyme.
2. The binding of the substrate stimulates the enzyme to change its shape and fit more tightly around the substrate.
3. The active site of the enzyme is now in close proximity to the substrate and breaks the chemical bonds of the substrate to form the new enzyme-product complex.
4. Finally, the enzyme releases the products of the reaction. The free enzyme is then ready to bind to another molecule of the substrate and run through the catalytic cycle once again.

Factors Affecting Enzyme Activity

When you think about enzyme activity, keep in mind that most enzymes are chemically proteins. Remember that the tertiary structure of a protein is vital for its biological functions. With this concept in mind, it's easy to see why the activity of an enzyme may be affected by a change in the conditions that can disrupt the tertiary structure of its protein chains.

These conditions include temperature, pH, change in substrate concentration, or the binding of certain chemicals that regulate its activity. Let's understand more about these factors in detail.

Enzyme kinetics is one of the most tested topics in biology exams. Focus on understanding V_{max} , K_m , and how competitive vs. non-competitive inhibitors affect these values. Drawing Lineweaver-Burk plots can help you visualize the differences quickly.

Temperature and pH

Enzymes usually function in a narrow range of temperature and pH. Scientists have discovered that every enzyme demonstrates its highest level of activity at a particular temperature and pH known as the **optimum temperature** and **optimum pH**. The enzyme activity steadily declines both below and above the optimum value.

At low temperatures, the enzyme is preserved in a temporarily inactive state and regains its lost activity when you raise the temperature back to normal. On the other hand, high temperature destroys enzymatic activity completely because the enzyme, being a protein, gets denatured by heat. Once the enzyme protein gets denatured, it remains inactive even if the temperature is brought down later. This is an irreversible change.

Similarly, a rise or fall in pH above or below the optimum reduces enzyme activity. Some enzymes act best in an acidic medium and others do so in an alkaline medium. For instance, pepsin in your stomach works optimally at a pH of about 2, while trypsin in the small intestine prefers a pH close to 8.

Concentration of substrate

With an increase in substrate concentration, the velocity of the enzymatic reaction rises at first. The reaction ultimately attains a peak value of velocity (V_{max}) which is not exceeded by any further rise in the concentration of the substrate. The reason behind this phenomenon is that the enzyme molecules are fewer in number than the substrate molecules. After saturation of these molecules, there are no free enzyme molecules left to bind with the additional substrate molecules.

K_m or the **Michaelis constant** is a mathematical derivation or constant that indicates the substrate concentration at which the chemical reaction catalyzed by an enzyme attains half its maximum velocity. In other words, it tells you the concentration of substrate at which the enzyme is working at half of its V_{max} . A low K_m means the enzyme has a high affinity for its substrate, while a high K_m indicates lower affinity.

Inhibition of Enzyme Activity

Any substance that can bring down the velocity of an enzyme-catalyzed reaction is known as an **inhibitor**. **Reversible inhibitors** bind to enzymes via non-covalent bonds. Subsequent dilution of the enzyme-inhibitor complex leads to the dissociation of the reversibly-bound inhibitor and the enzyme regains its activity.

On the other hand, irreversible inhibition takes place when an inhibited enzyme fails to regain its activity upon dilution of the enzyme-inhibitor complex. Certain **irreversible inhibitors** act by forming covalent bonds with specific groups of enzymes. For example, a class of insecticides known as organophosphates produces neurotoxic effects by irreversibly binding to the catalytic

site of the enzyme **acetylcholinesterase**.

Let's now understand the concepts of reversible, irreversible, and allosteric inhibition in detail.

Reversible inhibition

In reversible inhibition, the inhibitor binds non-covalently to the enzyme. Furthermore, the enzyme inhibition can be reversed if the inhibitor is removed. Reversible inhibition is further subdivided into the following categories:

1. Competitive inhibition
2. Non-competitive inhibition

Competitive inhibition

In this type of inhibition, the inhibitor closely resembles the real substrate structurally and is regarded as a **substrate analogue**. It binds reversibly to the same site on the enzyme that the substrate normally occupies by competing with it for the same. You can reverse the effect of a competitive inhibitor by increasing the concentration of the substrate [S].

There is no effect on V_{max} during competitive inhibition; at a sufficiently high [S], V_{max} is attained even in the presence of the inhibitor. However, competitive inhibitors increase the apparent K_m for the given substrate. This means that in the presence of a competitive inhibitor, you need more substrate to achieve $1/2 V_{max}$. Examples of competitive inhibition include:

- the inhibition of succinic dehydrogenase by malonate and oxaloacetate
- the inhibition of alcohol dehydrogenase by ethanol in methanol poisoning
- the control of bacterial pathogens by **sulpha drugs** that compete with the substrate P-amino benzoic acid (PABA) for the active site of the enzyme

Non-competitive inhibition

Non-competitive inhibition takes place when the inhibitor and substrate bind at different sites of the enzyme. Here, the inhibitor can bind to either the free enzyme or the ES complex, preventing the reaction from taking place. The inhibitor has no structural resemblance to the substrate in this case.

Unlike competitive inhibitors, non-competitive inhibitors decrease the V_{max} of the reaction. You can't overcome non-competitive inhibition by increasing the [S]. Since these inhibitors don't interfere with the binding of the substrate to the enzyme, the K_m remains unaltered.

Heavy metal ions such as Ag^+ , Pb^{2+} , and Hg^{2+} can non-competitively inhibit enzymes by binding

to cysteinyl sulfhydryl groups.

Irreversible inhibition

Here, the inhibitor binds covalently to the enzyme and inactivates it in an irreversible manner. These inhibitors are usually toxic substances that poison enzymes. For example, **iodoacetate** is an irreversible inhibitor of certain enzymes such as **papain** and **glyceraldehyde 3-phosphate dehydrogenase**.

Iodoacetate combines with sulfhydryl (-SH) groups at the active sites of these enzymes and renders them inactive. Another good example of irreversible inhibition is **cyanide**, which can kill animals by inhibiting the enzyme **cytochrome oxidase**.

Suicide inhibition is a specialized form of irreversible inhibition where the original inhibitor (the structural analogue or competitive inhibitor) is converted to a more potent form by the same enzyme it was supposed to inhibit. The new inhibitor formed as a result binds irreversibly with the enzyme (in contrast to the original inhibitor that binds reversibly).

A good example of suicide inhibition is **allopurinol**, a drug that inhibits the enzyme **xanthine oxidase**. It is used in the treatment of gout.

Allosteric inhibition

Some enzymes possess additional sites besides the active site, known as **allosteric sites**. Such enzymes are known as **allosteric enzymes**. Certain substances referred to as allosteric modulators (effectors or modifiers) bind to the allosteric site and regulate the enzyme activity.

When a positive (+) allosteric effector binds to the allosteric site (known as the *activator site*), the enzyme activity is increased. On the other hand, a negative (-) allosteric effector can inhibit the enzyme activity by binding to the allosteric site (called *inhibitor site* in this case).

For example, **glucose-6-phosphate** is an allosteric inhibitor of the enzyme **hexokinase**. It plays an important role in feedback regulation during **glycolysis**.

Isoenzymes

Isoenzymes are multiple molecular forms of an enzyme, synthesized by different genes, occurring in the same organism, and having a similar substrate activity. More than a hundred known enzymes have been found to have isoenzymes. For example:

- Alpha-amylase of wheat endosperm has **16 isoenzymes**.
- Lactic acid dehydrogenase has **5 isoenzymes**.

- Alcohol dehydrogenase has **4 isoenzymes**.

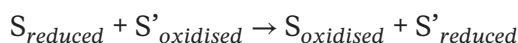
Isoenzymes are clinically significant because their levels in blood serum can help you diagnose tissue damage. For instance, different isoforms of lactate dehydrogenase (LDH) are elevated in heart, liver, or muscle injuries, making them valuable diagnostic markers.

Classification of Enzymes

With years of dedicated effort, biochemists have discovered, isolated, and studied thousands of enzymes. If you want to go deeper into enzyme biochemistry, check out these [best biology books](#). Most of these enzymes have been classified into different groups based on the type of reactions they catalyse. The current IUBMB classification divides enzymes into 7 classes, with each class further being divided into 4-13 subclasses and named accordingly by a four-digit number, which you'll learn about shortly.

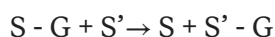
Oxidoreductases/dehydrogenases

These are enzymes that catalyse simultaneous oxidation and reduction (oxidoreduction) reactions between two substrates S and S' as shown below:



Transferases

They are enzymes catalysing a transfer of a group G (other than hydrogen) between a pair of substrates S and S' as seen below:



Examples of transferases include transaminases (transfer amino groups) and kinases (catalyse the phosphorylation of substrates by transferring phosphate groups, generally from ATP).

Hydrolases

They are enzymes that catalyse the hydrolysis of ester, ether, peptide, glycosidic, C-C, C-X or P-N bonds. Many hydrolases act on [carbohydrates](#) and other substrates. They facilitate the breakdown of larger molecules into smaller molecules with the addition of water.

Examples of hydrolases include amylases, proteases, lipases, nucleases, maltase, invertase, and other digestive enzymes. They are abundantly found in lysosomes.

Lyases

These are enzymes that catalyze the cleavage of substrates into two parts without involving hydrolysis for the removal of groups. They leave behind a double bond at the place from where the group was removed.

Aldolase, carbonic anhydrase, and decarboxylase are examples of lyases.

Isomerases

This class includes all enzymes that catalyze the rearrangement of the molecular structure of the substrate to form isomers. They facilitate the interconversion of optical, geometric, or positional isomers. Isomerase is a classic example of this class.

Ligases

Finally, ligases are enzymes that catalyze covalent bonding of two substrates to form a large molecule. They facilitate the joining of bonds such as C-O, C-S, C-N, and P-O with the help of energy obtained from ATP.

Examples of ligases are phosphoenolpyruvate carboxylase, RUBP carboxylase, and DNA ligase.

Translocases

Translocases are EC 7 enzymes that catalyze movement of ions or molecules across a membrane, or their separation within a membrane. ATP-driven pumps are useful examples. The transported substance and the energy-coupling reaction determine the subclass.

Older textbooks may list the original 6 classes. For current enzyme nomenclature, the [IUBMB translocase classification](#) explains the additional class.

Exoenzymes and Endoenzymes

Most enzymes remain and function within the cell, and are known as **intracellular enzymes** or **endoenzymes**. Some of them are dissolved in the cytoplasmic matrix, while others are bound to particles such as mitochondria, ribosomes, and chloroplasts. For example, respiratory enzymes that are required to convert lactic acid to CO₂ and water are present in the mitochondria.

However, certain enzymes leave the cell and function outside them. They are known as **extracellular enzymes** or **exoenzymes**. The main examples of such enzymes are digestive enzymes like salivary amylase, pepsin, and pancreatic lipase. It's worth noting that enzymes retain their catalytic activity even after they are extracted from the cells. This property has given them many

commercial applications in various industries.

Nomenclature of Enzymes

In 1961, the International Union of Biochemistry (IUB) appointed an Enzyme Commission (EC) to devise some basic principles for the classification and nomenclature of enzymes. The current IUBMB system includes 7 enzyme classes, including translocases. Each of these classes is subdivided into many subclasses, which are further divided into sub-subclasses. Finally, a four-digit Enzyme Commission (EC) number is assigned to each enzyme.

In each number, the significance of each digit is as follows:

1st digit - Class

2nd digit - Subclass

3rd digit - Sub-subclass

4th digit - Individual enzyme

Co-factors

Like other **proteins**, enzymes are composed of one or many polypeptide chains. However, scientists have observed that in a lot of instances, certain non-protein constituents known as **co-factors** are bound to the enzyme in order to render it catalytically active. In these cases, the protein portion of the enzyme is known as the **apoenzyme**.

Biochemists have identified three different types of co-factors:

- **Prosthetic groups**
- **Co-enzymes**
- **Metal ions**

Prosthetic groups

Prosthetic groups are organic compounds that, unlike the other two co-factors, are *tightly bound* to the apoenzyme. For example, in peroxidase and catalase (enzymes that catalyze the breakdown of H₂O₂ to water and oxygen), **haem** is the prosthetic group and it constitutes a part of the active site of the enzyme.

Co-enzymes

Co-enzymes are organic compounds like prosthetic groups, but their association with the apoenzyme is only transient. It usually takes place during the course of catalysis. Moreover, co-enzymes serve as co-factors in several different enzyme-catalyzed reactions. The vital chemical components of many coenzymes are vitamins. For example, the coenzymes nicotinamide adenine dinucleotide (**NAD**) and nicotinamide adenine dinucleotide phosphate (**NADP**) contain the vitamin niacin.

Interpreting K_m

In a simple Michaelis-Menten model, substrate concentration equal to K_m produces half of V_{max} . If K_m is 2 mM and V_{max} is 10 $\mu\text{mol/min}$, 2 mM substrate gives 5 $\mu\text{mol/min}$. At 8 mM, the predicted rate is $10 \times 8/(2 + 8) = 8 \mu\text{mol/min}$.

K_m is not universally identical to a binding dissociation constant. Its relation to affinity depends on the kinetic steps and assumptions. A competitive inhibitor can increase the apparent K_m without changing the limiting V_{max} in the ideal model, while other inhibition patterns require a different parameter interpretation.

The points to check in this example are:

- An enzyme accelerates an allowed reaction without changing its equilibrium free-energy difference.
- Temperature and pH effects are enzyme-specific.
- The current enzyme classification includes translocases as EC 7.

Check Your Understanding

Try the questions before reading the answers.

Question 1. What rate does the model predict at K_m ?

Answer. Half of V_{max} .

Question 2. Does adding more substrate always overcome every inhibitor?

Answer. No. The inhibition mechanism matters.

Practice Questions

Work each question before reading its solution. The set runs from direct recall and substitution to the applied questions that exams actually use to separate grades. The downloadable PDF includes these questions and their solutions.

Question 1. What is an enzyme, and by how much can one accelerate a reaction? Give the record-holder scale.

Solution. A biological catalyst, almost always a protein, that speeds a specific reaction without being consumed. Rate enhancements of 10^6 to 10^{17} -fold are documented: OMP decarboxylase converts a reaction with a half-life of 78 million years uncatalyzed into one taking milliseconds. Life at body temperature is impossible without this acceleration.

Question 2. How does an enzyme achieve its speedup thermodynamically, and what does it NOT change?

Solution. It lowers the activation energy by binding and stabilizing the transition state, offering an easier reaction path. It does not change ΔG , the equilibrium position, or the direction of reaction: an enzyme gets you to the same destination faster, never to a different destination. Both forward and reverse rates rise equally.

Question 3. Contrast the lock-and-key and induced-fit models of substrate binding.

Solution. Lock-and-key (Fischer, 1894) pictures a rigid active site precisely complementary to the substrate. Induced fit (Koshland, 1958) adds flexibility: binding reshapes the site around the substrate, like a glove tightening around a hand, often clamping catalytic groups into position. Induced fit is the modern default, with hexokinase's jaw-closing on glucose the textbook case.

Question 4. Why does enzyme activity collapse above an optimum temperature while ordinary reactions just keep accelerating?

Solution. Two competing effects: warming speeds molecular collisions (roughly doubling rates per 10°C), but past the optimum the enzyme's folded shape unravels, denaturation, destroying the active site geometry. Human enzymes peak near 37°C ; those of hot-spring archaea, used in PCR as Taq polymerase, survive near 100°C . The curve rises, peaks, and crashes.

Question 5. Sketch the effect of pH on pepsin (stomach) and trypsin (intestine), and explain the mechanism.

Solution. Pepsin peaks near pH 2, trypsin near pH 8: each is tuned to its workplace. pH shifts protonation of acidic and basic side chains in the active site and across the protein, altering charge pairings that hold the fold and grip the substrate. Away from the optimum, activity falls; far away, the enzyme denatures.

Question 6. Define V_{max} and K_m , and interpret a low K_m .

Solution. As substrate rises, rate saturates at V_{max} , where every enzyme molecule is busy. K_m is the

substrate concentration giving half of V_{max} . A low K_m means half-saturation at low substrate: high affinity, an enzyme that works well when its substrate is scarce. The Michaelis-Menten equation $v = V_{max}[S]/(K_m + [S])$ packages both.

Question 7. Distinguish competitive from noncompetitive inhibition, including their kinetic signatures.

Solution. A competitive inhibitor mimics the substrate and occupies the active site: raising substrate outcompetes it, so V_{max} is unchanged while apparent K_m rises. A noncompetitive inhibitor binds elsewhere and cripples catalysis regardless of substrate: V_{max} falls, K_m stays. Statins act competitively on HMG-CoA reductase; heavy metals act noncompetitively by attacking side chains anywhere.

Question 8. What is feedback inhibition, and why is the END product the logical inhibitor?

Solution. The final product of a pathway allosterically inhibits an early committed enzyme: isoleucine shuts down the first enzyme of its own 5-step synthesis. Inhibiting at the start avoids wasting intermediates and energy on a product already abundant, and releases automatically as the product is consumed. It is a thermostat wired into chemistry.

Question 9. Name the 6 international enzyme classes with 1 example each.

Solution. Oxidoreductases (alcohol dehydrogenase), transferases (kinases moving phosphate), hydrolases (amylase, proteases), lyases (decarboxylases), isomerases (phosphoglucose isomerase), ligases (DNA ligase). The EC number system files every known enzyme under these 6 verbs; a seventh, translocases, was added in 2018 for membrane movers.

Question 10. Explain how many drugs and poisons are enzyme stories, with 3 concrete cases.

Solution. Aspirin acetylates cyclooxygenase irreversibly, halting prostaglandin synthesis: pain relief by enzyme sabotage. Penicillin permanently inactivates the transpeptidase that crosslinks bacterial cell walls, killing bacteria while ignoring our enzyme-free wall-building (we have none). Nerve agents like sarin block acetylcholinesterase, so the neurotransmitter accumulates and muscles seize. Pharmacology is largely applied enzymology.

Metal ions

A large number of enzymes require **metal ions** for their biological activity. These ions form coordination bonds with side chains at the active site, and simultaneously also form one or more coordination bonds with the substrate. For example, **zinc** is an important cofactor for the proteolytic enzyme carboxypeptidase.

If you remove the co-factor from the enzyme, the catalytic activity is lost. This proves that co-factors play a pivotal role in the catalytic activity of the enzyme.

Given below is a list of important metal ions and the metal-containing enzymes (metalloenzymes) they are found in.

Metal ion(s)	Metalloenzymes
Fe ²⁺ , Fe ³⁺	Cytochrome oxidase, aconitase, catalase, peroxidase
Ca ²⁺	Succinate dehydrogenase, lipase
Mg ²⁺	Hexokinase, DNA polymerase, pyruvate kinase, phosphotransferase, enolase
Cu ²⁺	Cytochrome oxidase, tyrosinase
Co ²⁺	Peptidases, ascorbic acid oxidase
Mo	Nitrate reductase, dinitrogenase
Zn ²⁺	Carbonic anhydrase, Alcohol dehydrogenase, carboxypeptidase, LDH
Mn ²⁺	Arginase
Ni	Urease
Cl ⁻	Salivary amylase

What are enzymes and why are they important?

Enzymes are biological catalysts, mostly proteins, that speed up chemical reactions in living organisms without being consumed in the process. They are essential because virtually every metabolic reaction in your body, from digesting food to replicating DNA, depends on enzymes. Without them, these reactions would occur far too slowly to sustain life.

What is the difference between the lock and key model and the induced fit model?

The lock and key model (Emil Fischer, 1894) proposes that the enzyme's active site has a rigid, pre-shaped structure that perfectly matches the substrate, much like a key fits a specific lock. The induced fit model (Koshland, 1958) suggests that the active site is flexible and changes its shape to accommodate the substrate upon binding, similar to how a glove molds around a hand. The induced fit model is more widely accepted today because it accounts for the flexibility of enzyme structures observed in experimental studies.

How do temperature and pH affect enzyme activity?

Every enzyme has an optimum temperature and pH at which it functions most efficiently. Below the optimum temperature, enzyme activity decreases because molecular collisions slow down. Above the optimum, the enzyme protein denatures and loses its three-dimensional shape permanently. Similarly, extreme pH values disrupt the ionic and hydrogen bonds that maintain the enzyme's tertiary structure, reducing or eliminating its catalytic ability.

What is the difference between competitive and non-competitive inhibition?

In competitive inhibition, the inhibitor resembles the substrate structurally and competes for the same active site. You can overcome it by increasing the substrate concentration, and V_{max} remains unchanged while apparent K_m increases. In non-competitive inhibition, the inhibitor binds to a different site on the enzyme (not the active site), altering the enzyme's shape and reducing its catalytic efficiency. Increasing substrate concentration does not reverse non-competitive inhibition, V_{max} decreases, and K_m stays the same.

What are co-factors and why do enzymes need them?

Co-factors are non-protein molecules that bind to enzymes and are required for their catalytic activity. They come in three types: prosthetic groups (tightly bound organic molecules like haem), co-enzymes (loosely bound organic molecules often derived from vitamins, such as NAD and NADP), and metal ions (such as Zn^{2+} , Mg^{2+} , and Fe^{2+}). Without their respective co-factors, many enzymes cannot fold properly or catalyze reactions, and the protein portion alone (apoenzyme) remains inactive.

What is the Michaelis constant (K_m) and what does it tell you?

The Michaelis constant (K_m) is the substrate concentration at which an enzyme-catalyzed reaction proceeds at half of its maximum velocity (V_{max}). It is a measure of the enzyme's affinity for its substrate. A low K_m value means the enzyme reaches half of V_{max} at a low substrate concentration, indicating high affinity. A high K_m value means more substrate is needed to reach half of V_{max} , indicating lower affinity. K_m is unique for each enzyme-substrate pair and is widely used to compare enzyme efficiency.

What are isoenzymes and why are they clinically important?

Isoenzymes are multiple molecular forms of an enzyme produced by different genes within the same organism. They catalyze the same reaction but differ in their physical and chemical properties. Clinically, isoenzymes are valuable diagnostic markers. For example, different isoforms of lactate dehydrogenase (LDH) are elevated in heart attacks, liver disease, or muscle injuries. By measuring which specific isoenzyme is elevated in blood serum, doctors can pinpoint which tissue has been damaged.

How are enzymes classified and named?

The International Union of Biochemistry (IUB) classifies enzymes into 7 classes based on the reaction: oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases and translocases. Each enzyme receives a unique four-digit Enzyme Commission (EC) number. The first digit indicates the class, the second indicates the subclass, the third the sub-subclass, and the fourth identifies the specific enzyme. For example, EC 3.2.1.1 refers to alpha-amylase, a hydrolase that breaks down starch.