

Specificity of Enzymatic Reactions

Chapter Overview

In this chapter, we will explore how enzymes work, what distinguishes them from other catalysts, and why they are essential for life. First, we will explain why some reactions proceed slowly and how enzymes lower the activation energy. Next, we will show how physicochemical conditions of the environment (pH, temperature, ionic strength, redox state) and the presence of inhibitors or activators influence their activity. Finally, we will focus on **enzyme specificity**—the ability of enzymes to recognize only certain substrates or reactions—and demonstrate why this property has fundamental biological as well as pharmacological importance.

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1 Introduction

Around us we can observe many chemical reactions — some are obvious, such as the burning of wood, which is essentially the oxidation of cellulose releasing heat and light. Others are hidden, for example, the billions of chemical transformations that take place in our body every second. You may have wondered why these reactions proceed safely and simultaneously, without “getting in each other’s way”? One of the key answers is enzymes - biological catalysts that coordinate and accelerate specific reactions within the cell.

Enzymes are essential for reactions to proceed smoothly. Some reactions, although thermodynamically permissible, would not occur at all under physiological conditions (because of excessively high activation energy). Others would proceed far more slowly than is compatible with life. Enzymes enable these reactions and ensure that metabolic pathways function seamlessly.

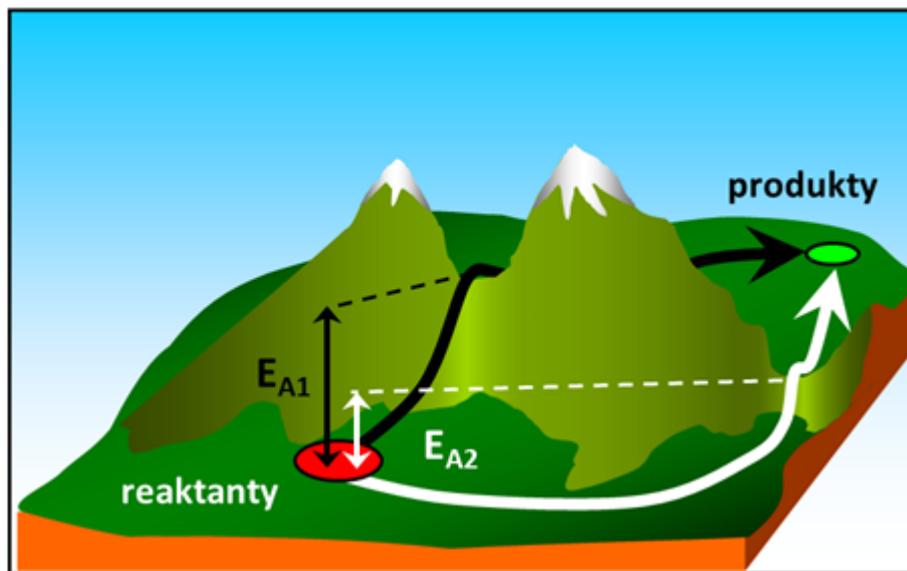
2 Why Some Reactions Proceed Slowly — Activation Energy

Enzymatic reactions proceed through the formation of a transient enzyme-substrate complex. The substrate (S) binds to the enzyme's active site (E), forming the ES complex. Within the complex, a chemical transformation takes place, producing the product (P). The product is released, while the enzyme remains intact and ready to process another substrate.

The first step (substrate binding) is usually rapid and reversible; the second step (product formation and release) is often slower and determines the overall reaction rate.

For substrates to be converted into products, the system must overcome the so-called activation energy: imagine it as a hill the reaction must "climb over." A higher activation energy means the molecules lack sufficient energy to reach the transition state, so the reaction is slow or does not occur at all. For example, ignition of cellulose: a flame provides the initial energy needed to start burning.

Catalysts, including enzymes, lower the activation energy by providing an alternative reaction pathway. The catalyst itself is not consumed during the reaction.



3 Enzymes — What Sets Them Apart From Other Catalysts

Enzymes are proteins (sometimes RNA — so-called ribozymes) with a specific active-site structure. Compared to simple inorganic catalysts, they have several distinctive features:

- Sensitivity to conditions — pH, temperature, ionic strength, and the presence of cofactors influence their activity.
- High specificity — they usually catalyse only one type of reaction or act on a very narrow group of substrates.

Enzymatic activity strongly depends on the physical and chemical conditions of the environment. The most important factors include pH, temperature, ionic strength and composition, redox state, and the presence of inhibitors or activators. The following sections describe each factor and its implications for both laboratory measurements and the biological function of enzymes.

3.1 Effect Of pH

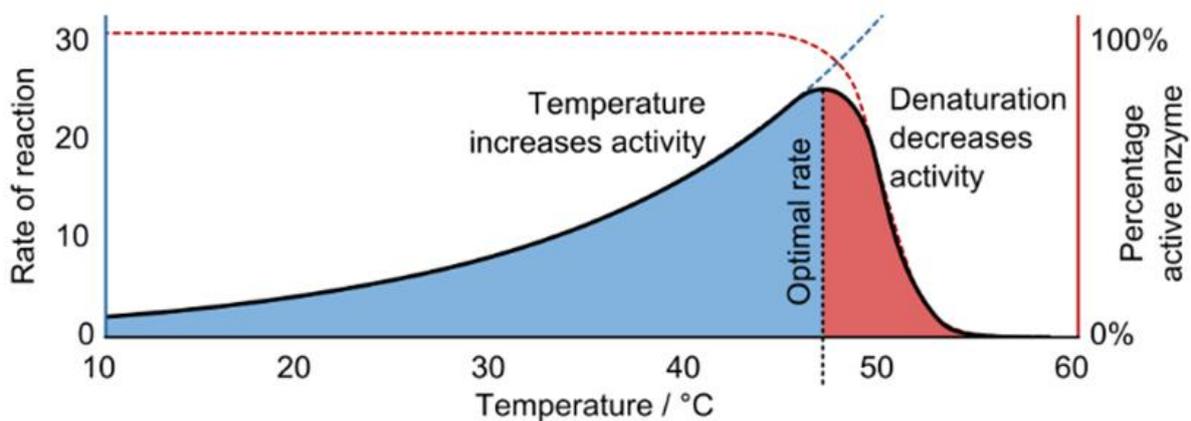
The pH of the environment strongly affects enzyme conformation. Even small pH shifts can alter ionization of amino acid side chains, thereby changing the enzyme's ability to recognize, bind, and convert the substrate. Substrate ionization can also be affected, weakening enzyme–substrate interactions. Extreme pH (strongly acidic or strongly alkaline) usually leads to denaturation of the protein structure and loss of catalytic activity.

Most enzymes have an optimum pH in the range 5–8, but there are important exceptions reflecting physiological localization. For example, pepsin functions best at pH 1.5–2.5 (stomach), while many intestinal enzymes are optimal in neutral to slightly alkaline conditions. Alkaline phosphatase has an optimum at pH 9.5–9.7, whereas acid phosphatase is active in acidic conditions.

Different enzymes may share similar names (e.g., multiple phosphatases) because they catalyse the same type of reaction or act on the same functional group, but differ in tissue localization, optimal conditions, structure, or regulation. Such enzyme variants allow organisms to perform similar chemical tasks in different cellular or organ environments.

3.2 Effect Of Temperature

Temperature affects both the rate of molecular collisions and the stability of the enzyme's protein structure. As temperature increases, reaction rates typically rise until an optimum is reached, usually between 35–45 °C for mammalian enzymes. At higher temperatures, thermal denaturation occurs and the reaction rate declines. At very low temperatures (near 0 °C), reactions are very slow; therefore, deep freezing (–20 °C or lower) is used for long-term preservation of biological material, where enzymatic reactions essentially stop.



(Source: https://upload.wikimedia.org/wikipedia/commons/thumb/d/d5/Q10_graph_c.svg/langcs-960px-Q10_graph_c.svg.png)

3.3 Ionic Strength, Buffer And Redox Conditions

Ionic strength influences electrostatic interactions between enzyme and substrate, as well as within the protein itself, affecting binding and conformation. For laboratory studies, buffers are always added to maintain stable pH and constant measurement conditions. The buffer type and concentration determine how well pH is maintained.

Redox conditions must also be considered: for enzymes containing disulfide bonds or redox cofactors, changes in redox state can cause loss of activity or switching between active and inactive forms.

A common cofactor is NADH, a typical coenzyme of dehydrogenases. It acts as an electron (and proton) donor — it is oxidized to NAD^+ during reductive steps and reduced back to NADH in oxidative steps. In practical enzymatic assays (e.g., lactate dehydrogenase activity), changes in NADH concentration can be directly monitored spectrophotometrically, as NADH absorbs strongly at 340 nm while NAD^+ does not. Thus, NADH decrease (when consumed as substrate) or increase (when formed as product) reflects the rate of the catalysed reaction.

3.4 Inhibitors And Activators

Enzyme inhibitors and activators fundamentally influence the rate and course of enzymatic reactions by altering substrate binding to the enzyme or modifying the catalytic activity of the active site.

- **Competitive inhibitors** compete with the substrate for the enzyme's active site. They can be "overcome" by increasing the substrate concentration—therefore, K_m increases (more substrate is needed to reach half of V_{max}), but the maximum reaction rate (V_{max}) remains unchanged.
- **Non-competitive inhibitors** bind outside the active site (e.g., at an allosteric site) and change the enzyme's shape, thereby reducing its maximum activity. In this case, K_m remains the same, but V_{max} decreases. A typical example is cyanide, which inhibits cytochrome c oxidase in the respiratory chain.
- **Enzyme activators** act in the opposite way—they facilitate substrate binding or stabilize the active conformation of the enzyme. Cofactors (e.g., metal ions such as Mg^{2+} , Zn^{2+} , or organic coenzymes such as NAD^+ , FAD) are essential for the proper function of many enzymes. Allosteric activators bind outside the active site and increase the enzyme's affinity for the substrate, thereby accelerating the reaction and enhancing the regulation of metabolic pathways.

These mechanisms have major practical importance—most drugs act precisely on the principle of enzyme inhibition or activation (e.g., ACE inhibitors in hypertension, statins in lipid metabolism disorders).

3.5 Enzyme Specificity

Enzyme specificity means that an enzyme acts only on certain substrates or catalyzes only a limited type of reactions. In other words, an enzyme “recognizes” only those molecules that match the shape and chemical properties of its active site. The active site is formed by the side chains of amino acids, and the substrate must not only fit spatially but also interact appropriately chemically.

Depending on scope, several types of specificity are distinguished:

- **Absolute specificity** – the enzyme acts only on one precisely defined substrate (e.g., urease cleaves only urea).
- **Group specificity** – the enzyme recognizes a certain functional group present on different molecules (e.g., alcohol dehydrogenase acts on the –OH group).
- **Bond specificity** – the enzyme is directed toward a specific chemical bond, such as ester or amide.
- **Stereospecificity** – the enzyme distinguishes between stereoisomers and acts only on one of them (e.g., L-amino acids vs. D-amino acids).

Two models help explain the mechanism of substrate recognition. The “**lock-and-key**” model describes a situation where the substrate precisely fits into the active site. The “**induced fit**” model shows that the enzyme partially adapts its shape after substrate binding, thereby stabilizing the complex.

Specificity has fundamental biological significance: it ensures the accuracy of metabolic pathways, prevents undesirable reactions, and allows fine regulation of metabolism. From a pharmacological perspective, enzyme specificity is crucial in drug design—the goal is to develop selective inhibitors or activators that affect only the target enzyme, thereby minimizing side effects.

Summary

Enzymes are biological catalysts that accelerate reactions by lowering activation energy, thus ensuring the smooth course of metabolism. Their activity depends on environmental conditions—pH, temperature, ionic strength, buffer, or redox state—and can be influenced by inhibitors or activators. Inhibitors slow down or block enzyme activity, while activators, often in the form of cofactors, enhance it. The key property of enzymes is specificity: each enzyme recognizes only certain substrates or reactions, ensuring the precision of metabolic pathways. Specificity also has essential clinical importance, as it enables targeted drug design and selective inhibitors.

Control Questions

1. What is activation energy, and what role do enzymes play in it?
2. How do enzymes differ from ordinary (inorganic) catalysts?
3. How does environmental pH affect enzyme activity? Give examples of enzymes with different pH optima.
4. What is the importance of ionic strength and buffer in studying enzymatic reactions?
5. Explain how competitive and non-competitive inhibition work and how their effects on K_m and V_{max} differ.
6. In what ways can cofactors or allosteric activators influence an enzymatic reaction?
7. What does enzyme specificity mean, and what types of specificity are distinguished?