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Review paper

Effect of pH, Temperature, and Concentration on Enzyme Activity: A Comprehensive Review

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ABSTRACT

Enzymes are biological catalyst they catalyze biochemical reactions under mild physiological conditions. There are some environmental and chemical factors which affect the activity and effectiveness of enzymes. In this review we tried to examine and explain how each of these parameters affects enzyme kinetics, conformational stability, and catalytic performance. With the help of this review, we also tried to explain and discuss the importance of these variables in the field of natural product and industrial applications which include pharmaceutical development, food technology, and biotechnology. A detailed understanding of these factors can enhance enzyme design, improve process efficiency, and expand enzyme use in non-natural conditions.

INTRODUCTION

Enzymes act as essential molecular agents within cells, driving a wide range of biochemical reactions that are necessary for life. They function by lowering the activation energy of reactions, allowing chemical processes to occur at a much faster rate than would be possible under normal biological conditions. A key characteristic of enzymes is their high specificity, meaning each enzyme typically interacts with particular substrates and catalyzes specific reactions. This

selectivity arises from the enzyme's unique three-dimensional structure, especially the shape and properties of its active site.

Enzyme activity, however, is dynamic rather than fixed. It is influenced by internal factors such as amino acid composition and structural flexibility, as well as by external environmental conditions. Among these external influences, pH, temperature, and substrate concentration are particularly important. Together, they determine reaction speed, substrate binding strength, and the overall stability of the enzyme. Changes in pH affect the

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ionization of amino acid residues within the enzyme, including those at the active site, which can alter binding and catalytic performance. Temperature affects molecular motion and reaction kinetics, with each enzyme operating best within a specific temperature range; beyond this range, structural damage or denaturation may occur. Substrate concentration also plays a major role in controlling reaction rate, especially as enzymes approach saturation, a relationship commonly explained by Michaelis–Menten kinetics. The interaction between enzymes and their surrounding environment is crucial not only for biological function but also for practical applications. Enzymes are widely used in medical diagnostics, such as biosensors and clinical assays, in pharmaceutical processes including drug synthesis, in agriculture to improve nutrient utilization and crop protection, and in environmental applications to aid in pollutant degradation. For these reasons, a detailed understanding of how pH, temperature, and substrate concentration influence enzyme activity is fundamental to biochemistry. This knowledge is also essential for optimizing enzyme use in industrial and technological contexts, enabling scientists and engineers to adjust conditions that enhance efficiency, stability, and overall performance across various fields.

2. Effect of pH on Enzyme Activity

2.1 Ionization and Active Site Dynamics

Enzymes are proteins composed of amino acid residues, many of which contain ionizable side chains. The ionization state of these groups affects the three-dimensional structure of the enzyme and, more critically, the configuration of its active site. A change in pH can alter the charge distribution within the enzyme, thereby affecting the binding of the substrate and the catalytic mechanism.

Most enzymes have an optimal pH range within which they function most effectively. Deviations from this optimum reduce catalytic activity and can eventually lead to denaturation.

2.2 Enzyme-Specific pH Optima

- **Pepsin**, a digestive protease secreted in the stomach, has an optimal pH of around 1.5–2.0.
- **Amylase**, found in saliva, works best at a near-neutral pH of around 6.7–7.0.
- **Urease**, from *Canavalia ensiformis*, operates optimally at pH 7.0–7.5.

The pH optimum often correlates with the enzyme's physiological environment, suggesting evolutionary adaptation to function in specific conditions.

2.3 Denaturation and Irreversible Inactivation

Extreme pH levels can break ionic and hydrogen bonds stabilizing the enzyme's tertiary structure, leading to irreversible denaturation. Denatured enzymes lose their functional shape, preventing substrate binding and catalytic action.

3. Effect of Temperature on Enzyme Activity

3.1 Kinetic Energy and Reaction Rates

Temperature directly influences molecular motion. At higher temperatures, substrates and enzyme molecules collide more frequently, generally increasing the rate of reaction. This relationship is described by the Arrhenius equation, where the rate constant (k) increases exponentially with temperature up to a critical point.

3.2 Optimal Temperature

Each enzyme has an optimal temperature at which it exhibits maximum catalytic activity. For most human enzymes, this is around 37°C, the normal body temperature. Beyond this optimum, enzyme



activity declines rapidly due to thermal denaturation.

3.3 Thermal Denaturation

- As temperature rises above optimal levels:
- Weak bonds such as hydrogen bonds and hydrophobic interactions break.
- The enzyme's structure begins to unfold.
- Active sites are disrupted, rendering the enzyme inactive.

Thermal denaturation is often irreversible.

3.4 Thermostable Enzymes

In contrast to mesophilic enzymes, thermophilic enzymes from organisms like *Thermus aquaticus* can remain stable at 70–100°C. These enzymes are invaluable in PCR (Polymerase Chain Reaction) and high-temperature industrial processes due to their robustness.

3.5 Temperature Coefficient (Q10)

The Q10 value expresses how the rate of a biological process changes with a 10°C increase in temperature. For most enzymatic reactions, Q10 is around 2, meaning the reaction rate doubles with every 10°C increase within the permissible range.

4. Effect of Substrate Concentration on Enzyme Activity

4.1 Michaelis-Menten Kinetics

The dependence of enzyme activity on substrate concentration is classically described by the **Michaelis-Menten equations**:

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

Where:

- v is the initial reaction rate,
- V_{max} is the maximum rate,
- $[S]$ is substrate concentration,

- K_m is the Michaelis constant (substrate concentration at half V_{max}).

4.2 Reaction Phases

- **Low [S]:** Enzyme activity increases linearly with [S] (first-order kinetics).
- **Intermediate [S]:** Reaction rate begins to plateau.
- **High [S]:** Enzymes become saturated, and rate reaches V_{max} (zero-order kinetics).

4.3 K_m as Affinity Indicator

A low K_m value suggests that the enzyme has a strong affinity for its substrate, whereas a higher K_m indicates weaker interaction. By determining K_m and V_{max} , researchers can effectively compare the catalytic efficiency of different enzymes.

4.4 Enzyme Inhibition at High Substrate Levels

At very high substrate concentrations, certain enzymes show substrate inhibition. In this situation, the excess substrate binds to allosteric sites on the enzyme, which interferes with normal activity and lowers catalytic efficiency.

5. Combined Effects of pH, Temperature, and Concentration

In biological systems, these parameters are closely linked rather than acting independently. For example, an increase in temperature can make an enzyme more responsive to changes in pH. Likewise, the optimal pH of an enzyme may vary with temperature, and enzyme–substrate interactions can differ under different ionic conditions.

Studies that examine multiple factors together often uncover such complex relationships. Because of this, enzyme engineering focuses on developing catalysts that can maintain activity

over a wide range of conditions, especially for industrial applications

6. Applications in Industry and Research

6.1 Biotechnology and Industrial Enzymes

- **Detergents** use alkaline proteases that function at high pH and temperature.
- **Food processing** relies on pectinases and cellulases optimized for specific pH-temperature conditions.
- **Textile and paper industries** use thermostable enzymes for fabric treatment and pulp processing.

6.2 Medical and Diagnostic Uses

- **Enzymes in biosensors**, such as glucose oxidase, must be stable under physiological conditions.
- **Enzyme replacement therapies** require enzymes with appropriate pH and thermal profiles for human tissues.

6.3 Agriculture

- Enzyme formulations in animal feed enhance digestion under specific pH and temperature conditions of the gut.

7. Strategies for Enhancing Enzyme Performance

To improve enzyme utility across varying conditions:

- **Protein engineering** modifies amino acid residues to enhance thermal or pH stability.
- **Immobilization techniques** anchor enzymes to solid supports, improving resistance to temperature and pH extremes.
- **Directed evolution** uses mutation and selection to evolve enzymes with desired kinetic properties.

CONCLUSION

The catalytic activity of enzymes depends strongly on factors such as pH, temperature, and substrate concentration. These factors influence enzyme structure, function, and efficiency both individually and in combination. A clear understanding of these parameters is essential in biochemistry and is also important for applications in industry, pharmaceuticals, and clinical research. Future studies are expected to further investigate the molecular basis of enzyme behavior under varying conditions and to develop tailored biocatalysts for demanding environments. Progress in computational biology, enzyme engineering, and synthetic biology is likely to support the creation of enzymes with improved performance, offering benefits across fields ranging from energy production to health care.

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