Chapter 5: Effect of pH on enzyme activity

Introduction

The **H^+ ion** is the general effector of enzymatic reactions. The concentration of H^+ determines the pH of the medium, which affects both the **kinetic parameters** of enzymes and the **conformational parameters** of proteins, as they are highly sensitive to pH variations.

Measuring many enzymatic activities as a function of pH results in curves that pass through a maximum, indicating the existence of an **optimum pH**. Consequently, the optimal pH has been determined for many enzymes.

pH can exert various effects, including:

- The ionization of the residues of enzyme, substrate, and product residues
- The tertiary structure of proteins and thus enzyme stability
- The binding of the substrate to the enzyme, impacting the formation of the enzyme-substrate complex
- An effect on the catalytic activity itself

1. Effect of pH on enzyme conformation

The different **ionizable chemical groups** present in a protein can be either **protonated or deprotonated** depending on the pH. It is common for enzymes to **denature** in highly acidic or, conversely, highly alkaline environments. This effect leads to a change in the **three-dimensional structure**, and the loss of activity is partly due to a variation in the **overall charge**.

The action of pH (H^+ or OH^-) often causes to **reversible denaturation**. However, at **extreme pH values**, denaturation becomes **irreversible**. The structural change caused by pH, leading to loss of catalytic activity, is sometimes due to the protonation or deprotonation of a **single group** within the protein.

A classic example is **trypsin**, where the active structure involves an **electrostatic interaction** (salt bridge) between COO^- and NH_3^+.

2. Effect of pH on Kinetic Parameters

pH affects the kinetic parameters ($\mathbf{V_{max}}$ and $\mathbf{K_M}$) of the reaction. This may result from the ionization of the enzyme, the substrate, or both molecules. In kinetic studies, substrate ionization (if it occurs) is generally not taken into account; only the ionization of the enzyme is considered.

Most pH effects on enzymatic reactions result from a simultaneous impact on both $\mathbf{V_{max}}$ and $\mathbf{K_M}$. It appears that only one ionic form of the enzyme (or a specific set of ionic forms) is **catalytically active**; this is the **EH form** (Figure 1).

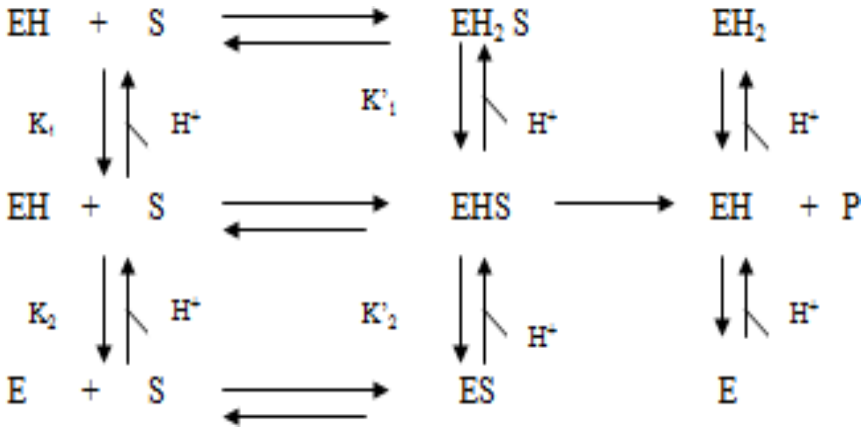

Figure 1. Simplified scheme involving a single intermediate complex (the Michaelis complex, EHS)

- **K_1:** Protonation constant of the free enzyme.
- **K_2:** Deprotonation constant of the free enzyme.
- **K'_1:** Protonation constant of the ES complex.
- **K'_2:** Deprotonation constant of the ES complex.

K_1, K_2, K'_1, and K'_2 represent the different **ionization constants** of the free enzyme and the Michaelis complex. Only the **EHS form** yields the product.

The reaction rate (velocity) is proportional to the quantity of the intermediate complex. To study the catalytic behavior of an enzyme as a function of pH, we work with a **saturating substrate concentration**; we therefore measure the **apparent $\mathbf{V_{max}}$**. This is a $\mathbf{V_{max}}$ that is only apparent: it represents the highest measurable velocity under those specific conditions. $\mathbf{V_{max}}$ is given by the following equation:

$$\boldsymbol{V}\max(\mathrm{pH})) = \boldsymbol{Vmax}\frac{\mathbf{1}}{\mathbf{1}+\frac{[\boldsymbol{H}+]}{\boldsymbol{K'1}}+\frac{\boldsymbol{K'2}}{[\boldsymbol{H}+]}}$$

2.1. Effect of pH on Vmax: (Vmax = f(pH))

The study of V_{max} variations as a function of pH allows for the determination of **K'₁** and **K'₂**. The method used is the **Dixon Plot** (log V_{max} as a function of pH, with pH = log $[H^+]$). The experimental curve is tangent to three straight-line segments with respective slopes of **+1, 0 and −1** (Figure 2).

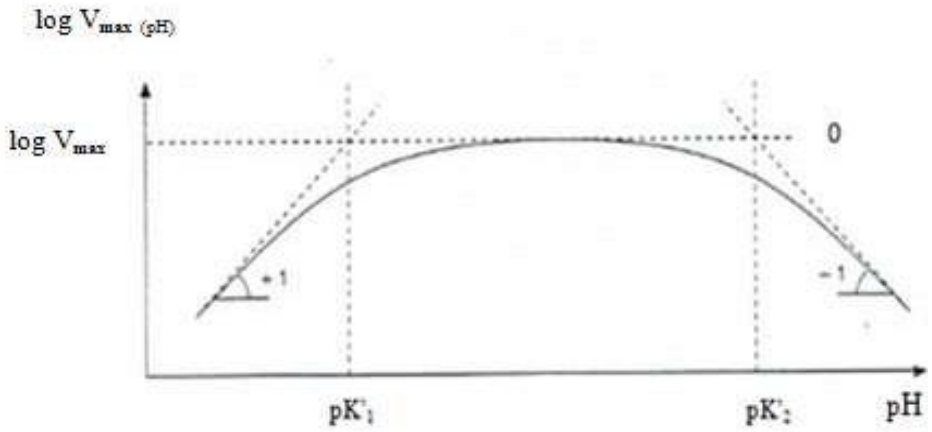

Figure 2. Graphical representation of log V_{max} as a function of pH

If **pK'₁** and **pK'₂** are sufficiently separated, the intersections of these three straight-line segments allow for the determination of **pK'₁** and **pK'₂**. Thus, V_{max} depends on the **ionization of the Michaelis complex**.

The advantage of the **Dixon method** is that it allows the determination, at extreme pH values, of the **number of groups** whose protonation or deprotonation leads to the loss of enzymatic activity.

2.2. Effect of pH on K_M (K_M = f(pH))

The value of K_M as a function of pH shows a more complex variation, since it depends on the ionization pK values of groups in both the **free enzyme** (**pK₁, pK₂**) and the **Michaelis complex** (**pK'₁, pK'₂**). The kinetic parameters are given by the following equation:

$$\boldsymbol{KM}\ (\mathrm{pH})) = \boldsymbol{KM}\ \frac{\mathbf{1} + \frac{[\boldsymbol{H}+]}{\boldsymbol{K1}} + \frac{\boldsymbol{K2}}{[\boldsymbol{H}+]}}{\mathbf{1} + \frac{[\boldsymbol{H}+]}{\boldsymbol{K'1}} + \frac{\boldsymbol{K'2}}{[\boldsymbol{H}+]}}$$

Assuming pK = log K_M. if pK_1 and pK'_1 are sufficiently separated from pK_2 and pK'_2, the expression of K_M (pH) simplifies in both acidic and alkaline media (Figure 3) as follows:

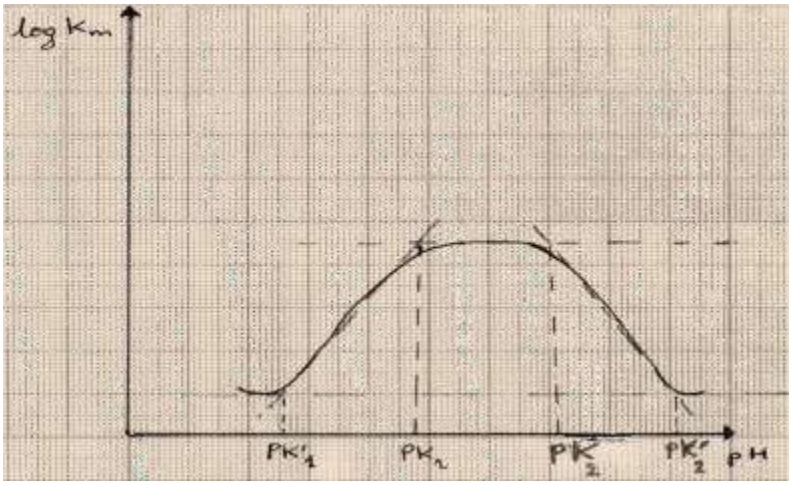

Figure 3. Graphical representation of log K_M as a function of pH

2.3. Effect of pH on the (Vmax/Km) ratio

(V_{max}/K_M) as a function of pH depends only on the ionization of the free enzyme, and the (V_{max}/K_M) curve as a function of pH is shown in Figure 4. The equation is as follows:

$$\boldsymbol{Vmax/KM}\ (\mathrm{pH})) = \boldsymbol{Vmax/KM}\frac{\mathbf{1}}{\mathbf{1}+\frac{[\boldsymbol{H}+]}{\boldsymbol{K1}}+\frac{\boldsymbol{K2}}{[\boldsymbol{H}+]}}$$

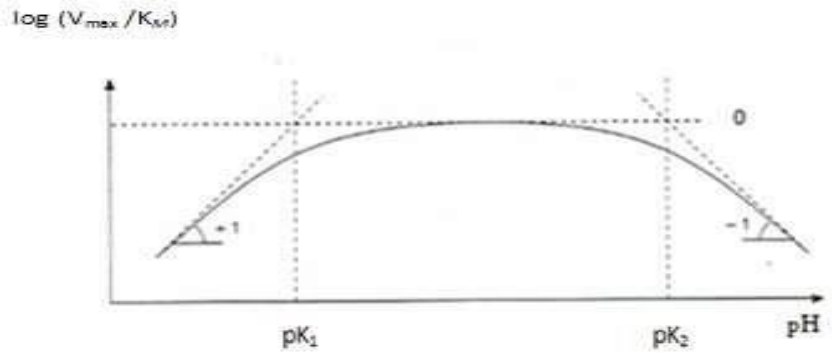

Figure 4. Graphical representation of log (V_{max}/K_M) as a function of pH