Chapter 6: Effect of temperature on enzymatic activity

1. Basic thermodynamic concepts

Thermodynamics is the study of a system as it evolves based on mechanical and thermal exchanges with the external environment (Figure 1).

- **H**: **(Enthalpy):** Enthalpy is a **state function** expressed in Joules (J). The term originates from Greek and refers to the heat. It represents the internal heat contained in a molecule.
- **ΔH (Enthalpy Change)** or change in enthalpy, which represents the heat variation between the **system** where the reaction takes place (the studied part) and the **surrounding** environment (the remaining part).

Chemical reactions are classified into two categories:

- **Exothermic**, where heat is released into the surroundings, so ΔH is negative. This is the most common case.
- **Endothermic**, where the system absorbs heat from the surroundings, resulting in a positive enthalpy change(ΔH).

Example:

$C + O_2 \longrightarrow CO_2$

With heat release, ΔH= - 393,51 kj

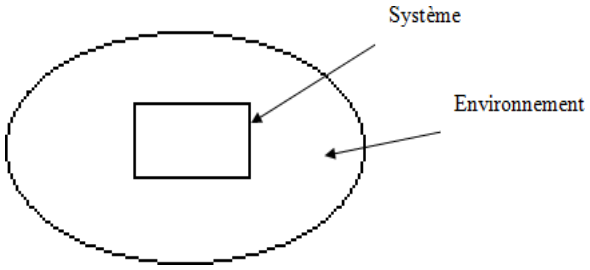


Figure 1. Mechanical and thermal exchanges between the system and the external environment

ΔG: change in free energy, it represents the energy that the system is free to use to perform work.

Consider the following reaction:

$S \rightarrow P$

S: contains an amount of energy called H_S.
P: contains an amount of energy called H_P

$\Delta H = H_P - H_S$ (Figure 2)

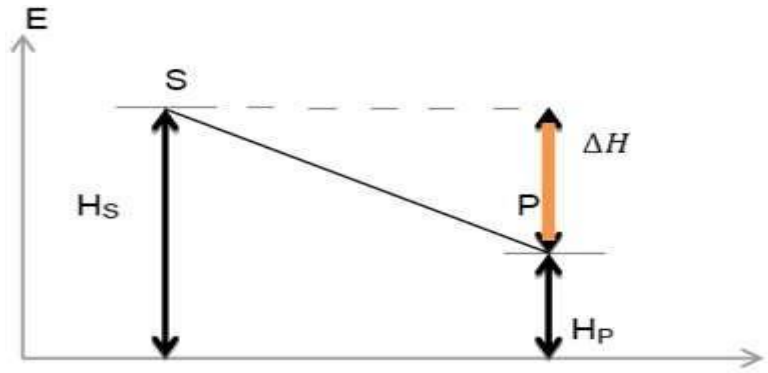


Figure 2. Energy balance of a reaction

In thermodynamics, a **process** is defined as the variation of one of the system's properties. When **heat**, **work**, or **matter** are exchanged between the system and its **surroundings**, the system evolves from its initial **equilibrium state** to a new equilibrium state.

2. Effect of Temperature on Equilibrium Constants

Consider the following reaction:

$$aA + bB \rightleftharpoons cC + dD$$

$$\Delta G = \Delta G^0 + RT \ln K$$

When equilibrium is reached: $\Delta G=0$, therefore:

$$\Delta G^0 = - RT \ln K$$

- **R:** Universal gas constant (R = 1.987 kcal/mol).
- **T:** Temperature in Kelvin (K°).
- **K :** Equilibrium constant.

The variations in free energy, entropy, and enthalpy are related by the following equation (**Gibbs function**):

$$\Delta G^0 = \Delta H0 - T\,\Delta S^0$$

ΔG^0: Standard Free Energy change (or **Gibbs Free Energy**). It is associated with the principle of the evolution of physicochemical systems when working at constant pressure and volume, which is the case for most enzymatic reactions studied, and all compounds are in their **standard state**.

ΔS: Entropy, a term introduced by Rudolf Clausius in 1865 from a Greek word meaning "transformation". It describes the **degree of disorder** (disorganization) of the system. Entropy

measures the degree of dispersion of energy (thermal, chemical, or electrical) within a system. The study of ln K variations as a function of temperature allows for the calculation of thermodynamic parameters using the **van 't Hoff equation** (**Figure 3**):

$$\boldsymbol{ln\ K} = -\frac{\Delta H^\circ}{\boldsymbol{R}} \cdot \frac{\mathbf{1}}{\boldsymbol{T}} + \frac{\Delta S}{\boldsymbol{R}}$$

The graphic representation is

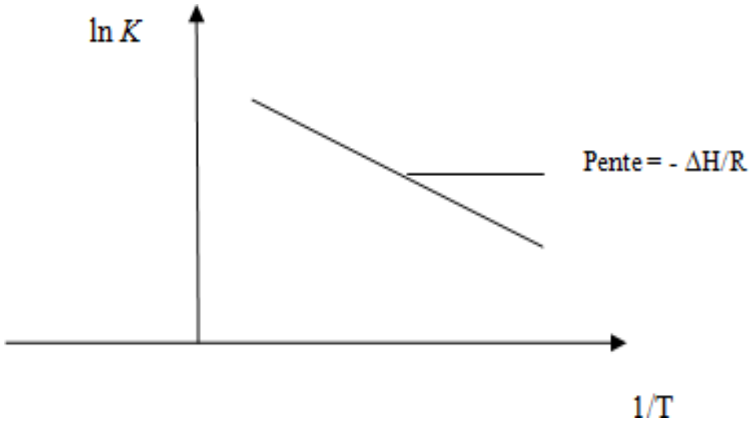


Figure 3. Representation of the variation of ln K as a function of 1/T

2.1. Effect of temperature on reaction rate

2.1.1. Definition of Q_{10}

Q_{10}: This is the temperature coefficient (or growth factor) of an enzymatic reaction increases (rises) when the temperature increases by 10°C. This factor typically increases by a magnitude of **2 to 3** for every 10°C interval.

2.1.2. Theories explaining reaction mechanisms

There are two theories that explain reaction mechanisms: **Collision Theory** and **Absolute Rate Theory** (also known as Transition State Theory).

a. Collision Theory

This is a stochastic (random) theory. The **activation energy** is provided by collisions between reacting molecules. While the total number of collisions increases with which does not fully explain the increase in reaction rate. Instead, it is the **energy of the collisions** increases s significantly, which is the primary cause of the accelerated reaction rate.

The fraction of molecules whose energy is higher than ΔG* is calculated by the **Boltzmann equation**:

$$n = e^{-\Delta G*/RT}$$

Where ΔG^* is the kinetic activation energy, also called Ea^* (activation energy). The reaction rate is given by the **Arrhenius law**:

$$v = p \cdot Z \cdot e^{-Ea/RT}$$

- **p**: The steric factor (fraction of molecules leading to a successful reaction).
- **Z**: Represents the number of collisions per unit time and volume at a given temperature.
- **P. Z** : This is a non-measurable factor and is replaced by the factor **A**, designated as the **pre-exponential factor** (or frequency factor), known as a system constant

In logarithmic form, the equation is written as follows:

$$\mathbf{Ln\ v = ln\ A - E_a^* / RT}$$

This equation allows us to plot a linear curve: ln v as a function of 1/T (Figure 4).

The graphic representation is

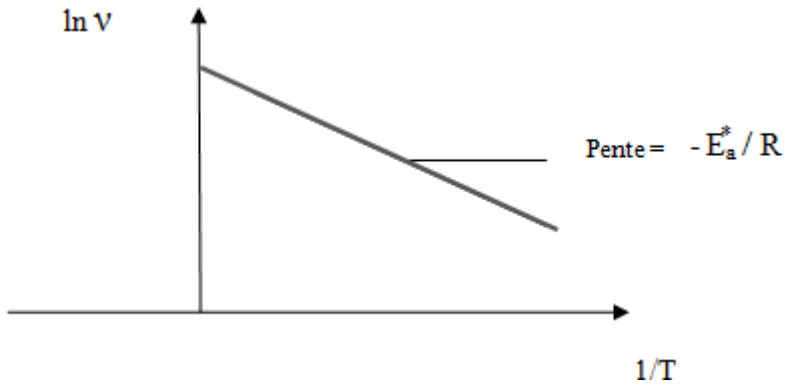


Figure 4. Plot of ln v as a function of 1/T

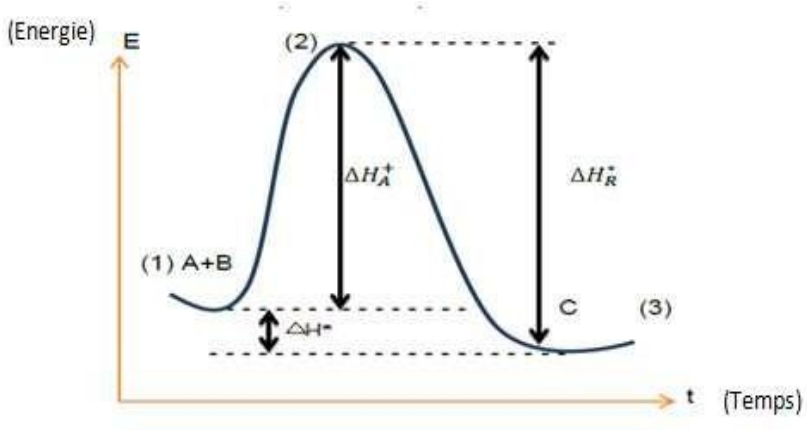
$\mathbf{E_a^*}$ is related to the activation enthalpy (ΔH^*) by the following equation:

$\mathbf{\Delta H^* = E^*a - RT}$ (From $\mathbf{E^*a}$, we can calculate $\mathbf{\Delta H^*}$).

b. Absolute Rate Theory (Transition State Theory)

This theory involves an intermediate known as the **activated complex**, which exists in equilibrium with the reacting species. The reaction rate depends on the number of molecules that possess a sufficient energy state. The development of this theory shows that the reaction rate is proportional to the concentration of the activated complex, also called the **transition state** complex.

The reaction: A + B → C can be represented as follows:



(1)Initial state
(2) Intermediate state
(3) Final state

ΔH* : Enthalpy of activation (Gibbs free enthalpy) change of the reaction.

In enzymatic reactions, E + S forms several complexes:

$$E + S \rightleftarrows ES \rightleftarrows ES^* \rightleftarrows EY \rightleftarrows EY^* \rightleftarrows EP \rightleftarrows EP^* \rightleftarrows E + P$$

The reaction velocity is proportional to the concentration of the **activated complex**, and the proportionality factor is identical for all reactions. Therefore:

$$\boldsymbol{v} = \frac{kT}{\boldsymbol{h}} \qquad \text{and} \qquad \boldsymbol{Kr} = \frac{kT}{\boldsymbol{h}}\, \boldsymbol{K}*$$

- **k**: Boltzmann constant :1.38 10^{-23} J/K
- **h**: Planck constant: 6.626 10^{-34} J.s

Hence:

$$\boldsymbol{\Delta G^* = -RT \ln K^*}$$

$$\boldsymbol{\Delta G^* = \Delta H^* - T\,\Delta S^*}$$

From the variations of ln K* as a function of temperature, ΔH* and ΔS* can be determined.

2.2. Thermal Denaturation of Enzymes

Thermal denaturation often follows first-order kinetics:

$$Ea \xrightarrow{k_i} Ei$$

$\ln(E/E_0) = -k_i t$ where ki is the inactivation constant.

- **E_a** : active enzyme
- **E_i** : inactive enzyme
- **E**: enzymatic activity at time t
- **E_0**: enzymatic activity at time t_0

In practice, the residual activity of the enzyme is measured after different heating times. The graphical representation of: $\ln (E/E_0) = f(t)$ gives a straight line with slope $-k$ (Figure 5).

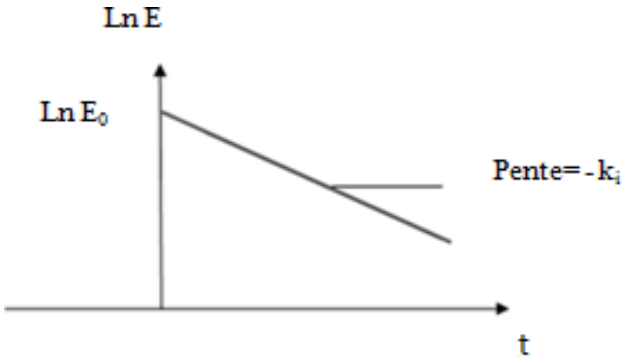


Figure5. Courbe de ln E en fonction du temps t. It represents the thermal inactivation kinetics of the enzyme