ENZYME KINETICS AND REGULATION OF ENZYME ACTIVITIES

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BIOMEDICAL IMPORTANCE

- A thorough and balanced set of **enzyme activities** is required for maintaining homeostasis.
- Enzyme kinetics, ***the quantitative measurement of the rates of enzyme-catalyzed reactions and the systematic study of factors that affect these rates***, constitutes a central tool for the analysis, diagnosis, and treatment of the enzymatic imbalances that underlie various human diseases.
- For example, in the blood, the appearance or a surge in the levels of particular enzymes serve as clinical indicators for pathologies such as myocardial infarctions, acute pancreatitis, and damage to the liver.

FREE ENERGY CHANGE DETERMINES THE DIRECTION & EQUILIBRIUM STATE OF A CHEMICAL REACTION

- The Gibbs free energy change ΔG (free energy or Gibbs energy) describes in quantitative form both the *direction* in which a chemical reaction will tend to proceed and the concentrations of reactants and products that will be present at equilibrium state.
- ΔG_0 stands for the change in free energy that accompanies transition from the standard state to equilibrium.
- More useful biochemical term is $\Delta G_0'$, which defines ΔG_0 at pH 7.0.

- If the free energy of formation of the products is *lower* than that of the substrates, the signs of ΔG_0 and $\Delta G_0'$ will be *negative*, indicating that the reaction as written is favored in the direction left to right.
- Such reactions are referred to as **spontaneous.**
- The **sign** and the **magnitude** of the free energy change determine how far the reaction will proceed.

$$\boldsymbol{\Delta G_0 = -RT \ln K_{eq}}$$

For the reaction A + B $\longleftrightarrow$ P + Q

$$K_{eq} = \frac{[P][Q]}{[A][B]}$$

- Notice that, since ΔG_0 is a function exclusively of the initial and final states of the reacting species, it can provide information only about the *direction* and *equilibrium state* of the reaction.
- ΔG_0 is independent of the **mechanism** of the reaction, and provides no information concerning ***rates*** of reactions.
- Therefore, **although a reaction may have a large negative ΔG_0 or $\Delta G_0'$, it may yet take place at an insignificant rate**.

THE RATES OF REACTIONS ARE DETERMINED BY THEIR ACTIVATION ENERGY

- For a reaction to occur, the substrates undergoing the reaction need to be activated.
- If the energy levels of a substrate are plotted as the substrate is progressively converted to product, the curve will show a maximum energy level that is higher than that of either the substrate or the product.
- This high energy level occurs at the transition state.
- The difference in energy between the substrate and the transition state complex is called the **activation energy**.

- According to transition state theory, the overall rate of the reaction is determined by the number of molecules acquiring the activation energy necessary to form the transition state complex.
- **Enzymes increase the rate of the reaction by decreasing this activation energy**.
- They use different catalytic strategies, such as electronic stabilization of the transition state complex or acid-base catalysis, to obtain this decrease.
- The enzyme does not change the initial energy level of the substrates or the final energy level of the products.
- Therefore, **enzymes have no effect on *equilibrium constant***.

VARIOUS FACTORS AFFECT THE RATES OF ENZYME-CATALYZED REACTIONS

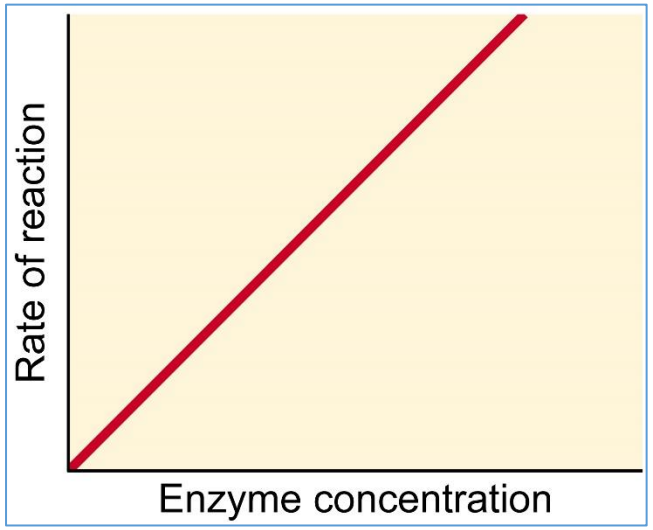
- Raising the **temperature** increases the rate of both uncatalyzed and enzyme-catalyzed reactions by increasing the kinetic energy and the collision frequency of the reacting molecules.
- However, for enzyme-catalyzed reactions, further elevation of the temperature results in a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme.
- The optimum temperature for most human enzymes is between 35 and 40°C.

VARIOUS FACTORS AFFECT THE RATES OF ENZYME-CATALYZED REACTIONS

- The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration, **pH**.
- Extremes of pH can also lead to denaturation of the enzyme, because the structure of the catalytically active protein molecule depends on the ionic character of the amino acid side chains.
- The pH at which maximal enzyme activity is achieved is different for different enzymes.

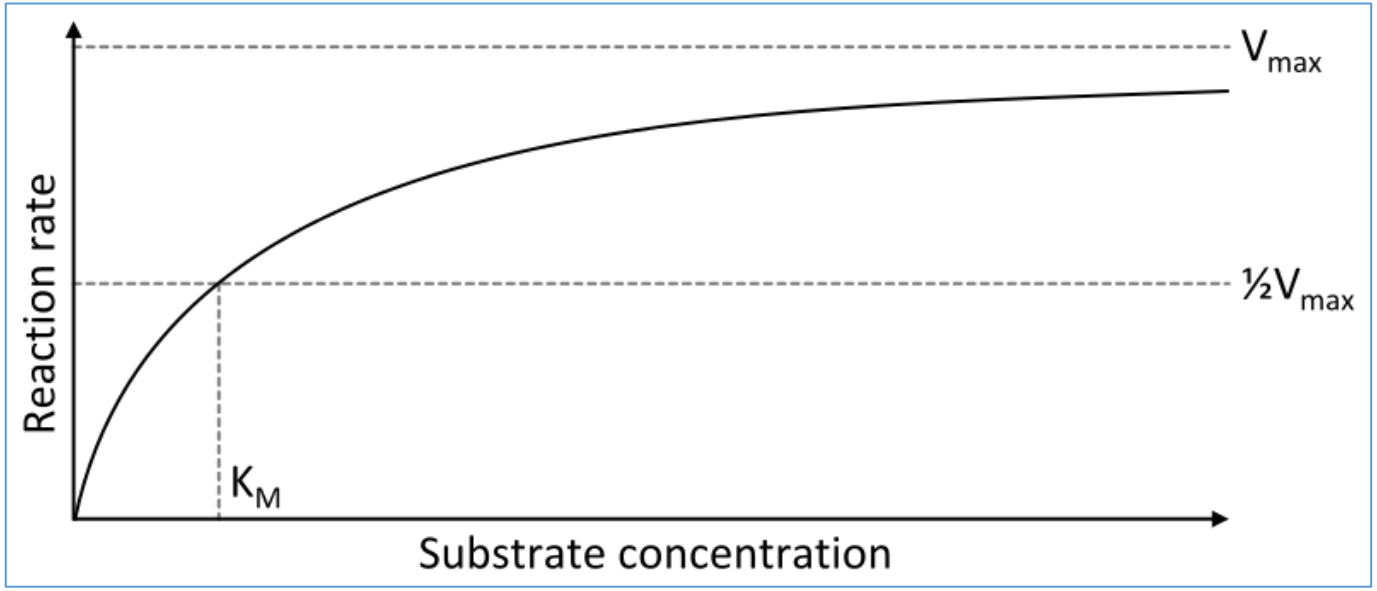
Relationship of velocity to enzyme concentration

- The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations.



SUBSTRATE CONCENTRATION AFFECTS THE REACTION RATE

- For a typical enzyme, as substrate concentration is increased, initial rate (v_i or v_o) increases until it reaches a maximum value V_{max}.
- When further increases in substrate concentration fail to increase v_i, the enzyme is said to be "saturated" with the substrate.



https://en.wikipedia.org/wiki/Michaelis-Menten_kinetics

Michaelis–Menten curve

The Michaelis-Menten Equation

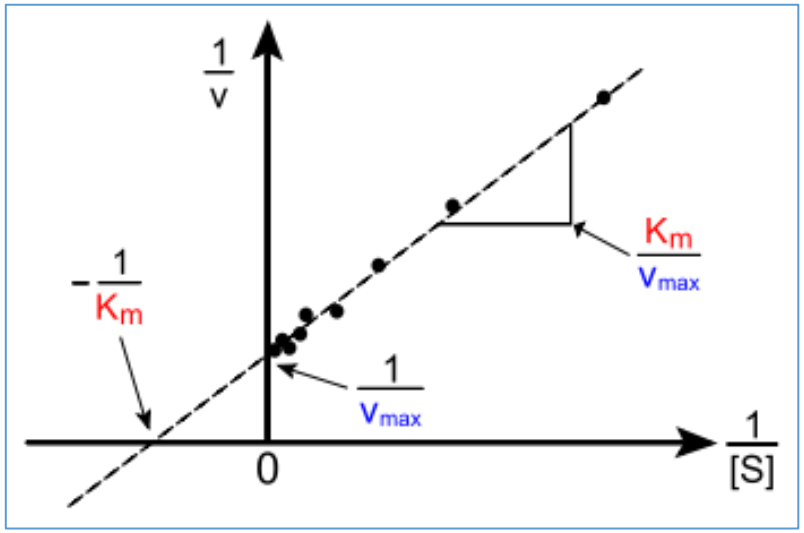
- The Michaelis-Menten equation illustrates in mathematical terms the relationship between initial reaction velocity v_i and substrate concentration [S], shown graphically in the preceding figure.

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

- The Michaelis constant $\boldsymbol{K_m}$ is the substrate concentration at which v_i is half the maximal velocity ($V_{max}/2$) attainable at a particular concentration of the enzyme.

A Linear Form of the Michaelis-Menten Equation Is Used to Determine K_m & V_{max} Exactly

$$\frac{1}{v_i} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



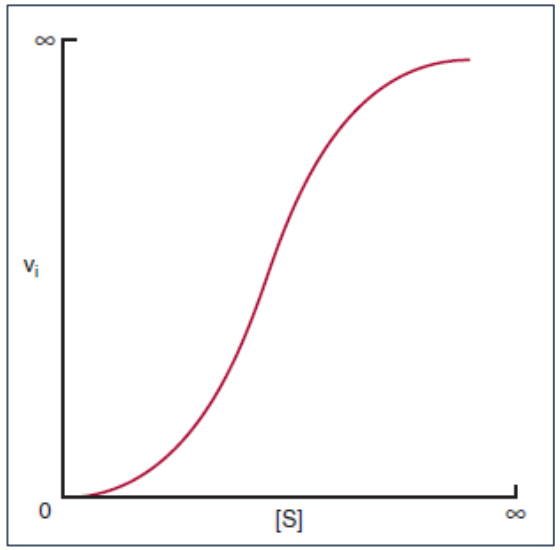
https://en.wikipedia.org/wiki/Lineweaver-Burk_plot

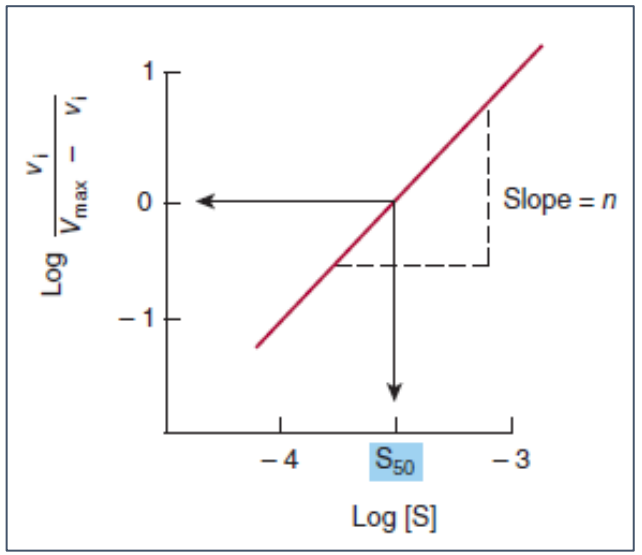
Double-reciprocal or Lineweaver-Burk plot

The Hill Equation Describes the Behavior of Enzymes That Exhibit Cooperative Binding of Substrate

- Cooperative behavior is an *exclusive* property of multimeric enzymes that bind substrate at multiple sites.
- For enzymes that display positive cooperativity in binding the substrate, the shape of the curve that relates changes in v_i to changes in [S] is sigmoidal.

$$\frac{\log v_i}{V_{max} - v_i} = n \log[S] - \log k'$$





INHIBITION OF ENZYME ACTIVITY

- Any substance that can diminish the velocity of an enzyme-catalyzed reaction is called an **inhibitor**.
- In general, irreversible inhibitors bind to enzymes through covalent bonds.
- Reversible inhibitors typically bind to enzymes through noncovalent bonds.
- Three types of reversible inhibition are:
  - Competitive inhibition
  - Noncompetitive inhibition
  - Uncompetitive inhibition

Competitive Inhibition

- The inhibitor binds reversibly to the same site that the substrate would normally occupy.
- Therefore, the inhibitor competes with the substrate for that site.

Competitive Inhibition

- **Effect on V_{max}:** The effect of a competitive inhibitor is reversed by increasing [S]. At a sufficiently high substrate concentration, the reaction velocity reaches the V_{max} observed in the absence of inhibitor.
- **Effect on K_m:** A competitive inhibitor increases the apparent K_m for a given substrate. This means that, in the presence of a competitive inhibitor, more substrate is needed to achieve $1/2V_{max}$.

Noncompetitive Inhibition

- Noncompetitive inhibition occurs when the inhibitor and substrate bind at different sites on the enzyme.
- The noncompetitive inhibitor can bind either free enzyme or the ES complex.

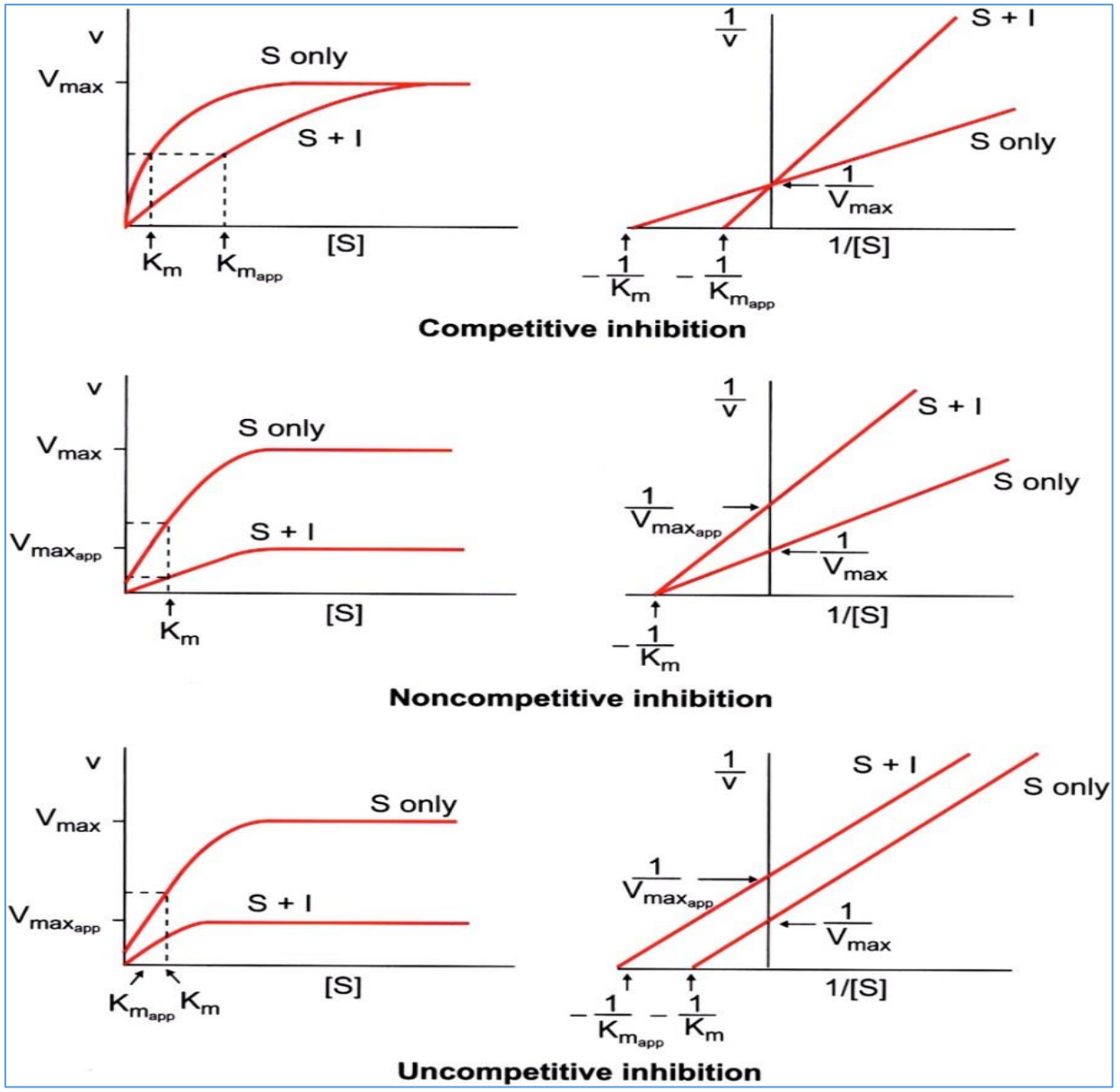
Noncompetitive Inhibition

- **Effect on V_{max}:** Noncompetitive inhibition cannot be overcome by increasing the concentration of substrate. Thus, noncompetitive inhibitors decrease the apparent V_{max} of the reaction.
- **Effect on K_m:** Noncompetitive inhibitors do not interfere with the binding of substrate to enzyme. Thus, the enzyme shows the same K_m in the presence or absence of the noncompetitive inhibitor.

Uncompetitive Inhibition

- An uncompetitive inhibitor binds at a site distinct from the substrate active site, but it binds only to the ES complex.

Types of Reversible Enzyme Inhibition



REGULATION OF ENZYMES

- In the body, thousands of various enzymes are regulated to fulfil their individual functions without waste of dietary components.
- Thus, with changes in our physiologic state, time of eating, environment, diet, or age, the rates of some enzymes must increase and others decrease.

- Changes in the rate of a metabolic pathway occur because at least one enzyme in that pathway, the **regulatory enzyme**, has been **activated** or **inhibited,** or the amount of enzyme has increased or decreased.
- Regulatory enzymes usually catalyze the **rate-limiting**, or slowest, step in the pathway, so that increasing or decreasing their rate adjusts the rate of the entire pathway.

Enzyme Regulation Mechanisms

- Substrate concentration (availability of substrate)
- Reversible inhibition
- Allosteric activators or inhibitors
- Covalent modification
- Protein–protein interactions
- Zymogen cleavage
- Changes in enzyme concentration
- Compartmentation of enzymes

REGULATION OF ENZYME ACTIVITY

- Allosteric modification
- Covalent modification
- Induction and repression of enzyme synthesis

Regulation of allosteric enzymes

- Allosteric enzymes are regulated by molecules called effectors that bind noncovalently at a site other than the active site.
- These enzymes are usually composed of multiple subunits, and the regulatory (allosteric) site that binds the effector may be located on a subunit that is not itself catalytic.
- The presence of an allosteric effector can alter the affinity of the enzyme for its substrate, or modify the maximal catalytic activity of the enzyme, or both.
- Effectors that inhibit enzyme activity are termed negative effectors, whereas those that increase enzyme activity are called positive effectors.

Regulation of enzymes by covalent modification

- Most frequent form of covalent modification is the addition or removal of phosphate groups from specific **serine**, **threonine**, or **tyrosine** residues of the enzyme.
  - **Phosphorylation and dephosphorylation**
  - **Response of enzyme to phosphorylation:**
    - Depending on the specific enzyme, the phosphorylated form may be **more** or **less** active than the unphosphorylated enzyme.

Induction and repression of enzyme synthesis

- Cells can regulate the amount of enzyme present by changing the rate of enzyme degradation or, more typically, the rate of enzyme synthesis.
- The increase (induction) or decrease (repression) of enzyme synthesis leads to an alteration in the total population of active sites.

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