ENZYMES

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OVERVIEW

- Virtually all reactions in the body are mediated by enzymes.
- They are protein catalysts (*except ribozymes which are RNAs with catalytic activity*) that increase the rate of reactions without being changed in the overall process.
- Each enzyme is assigned two names:
  - The first is its short, recommended name, convenient for everyday use.
  - The second is the more complete systematic name.

NOMENCLATURE

- The International Union of Biochemistry (IUB) developed an unambiguous system of enzyme nomenclature in which each enzyme has a unique name and code number that identify the type of reaction catalyzed and the substrates involved.
- For example:
  - L-lactate dehydrogenase (EC 1.1.1.27) known as LDH or LD
  - ATP:D-hexose 6-phosphotransferase (EC 2.7.1.1) known as hexokinase

CLASSIFICATION OF ENZYMES

- **1. Oxidoreductases**—enzymes that catalyze oxidations and reductions.
- **2. Transferases**—enzymes that catalyze transfer of moieties such as glycosyl, methyl, or phosphoryl groups.
- **3. Hydrolases**—enzymes that catalyze *hydrolytic* cleavage of C–C, C–O, C–N, and other covalent bonds.
- **4. Lyases**—enzymes that catalyze cleavage of C–C, C–O, C–N, and other covalent bonds by *atom elimination,* generating double bonds.
- **5. Isomerases**—enzymes that catalyze geometric or structural changes *within* a molecule.
- **6. Ligases**—enzymes that catalyze the joining together (ligation) of two molecules in reactions coupled to the hydrolysis of ATP.

PROPERTIES OF ENZYMES

- Enzymes are protein catalysts that increase the velocity of a chemical reaction, and are **not produced or consumed** during the reaction.
- Enzyme molecules contain a special pocket or cleft called the **active site**.
- The active site contains amino acid side chains that participate in substrate binding and catalysis.

PROPERTIES OF ENZYMES

- The substrate binds the enzyme, forming an enzyme-substrate (ES) complex.
- ES complex is converted to an enzyme-product (EP) complex that subsequently dissociates to enzyme and product.

PROPERTIES OF ENZYMES

- Enzyme-catalyzed reactions are greatly efficient, proceeding from 10^3-10^8 times faster than uncatalyzed reactions (**catalytic efficiency**).
- **Turnover number (k_{cat})**: It is equivalent to the number of substrate molecules converted to product in a given unit of time on a single enzyme molecule when the enzyme is saturated with its substrate.

PROPERTIES OF ENZYMES

Holoenzymes

- Some enzymes require molecules other than proteins for enzymatic activity.
- The term holoenzyme refers to the active enzyme with its nonprotein component.
- The enzyme without its nonprotein moiety is termed an **apoenzyme** and is inactive.

PROSTHETIC GROUPS, COFACTORS, & COENZYMES PLAY IMPORTANT ROLES IN IN ENZYMATIC CATALYSIS

- Many enzymes contain small molecules or metal ions that participate directly in substrate binding or in catalysis.
- These are **prosthetic groups**, **cofactors**, and **coenzymes**.
- Prosthetic groups are tightly integrated into an enzyme's structure.
- Cofactors associate reversibly with enzymes or substrates.

PROPERTIES OF ENZYMES

- Enzymes are highly **specific**, interacting with one or a few substrates and catalyzing only one type of chemical reaction.

- Enzyme **activity can be regulated**, that is, increased or decreased, in response to cellular need.

PROPERTIES OF ENZYMES

- Many enzymes are **localized** in specific organelles within the cell.
- Such compartmentalization serves to isolate the reaction substrate or product from other competing reactions.

ISOENZYMES

- Isoenzymes (also called isozymes) are alternative forms of the same enzyme that exist in different proportions in different tissues.
- Isoenzymes differ in amino acid composition and sequence and multimeric quarternary structure; mostly, but not always, they have similar (conserved) structures.
- Isoenzymes are a group of enzymes that catalyze the same reaction but have different enzyme forms and catalytic efficiencies.
- Isoenzymes are usually distinguished by their electrophoretic mobilities.

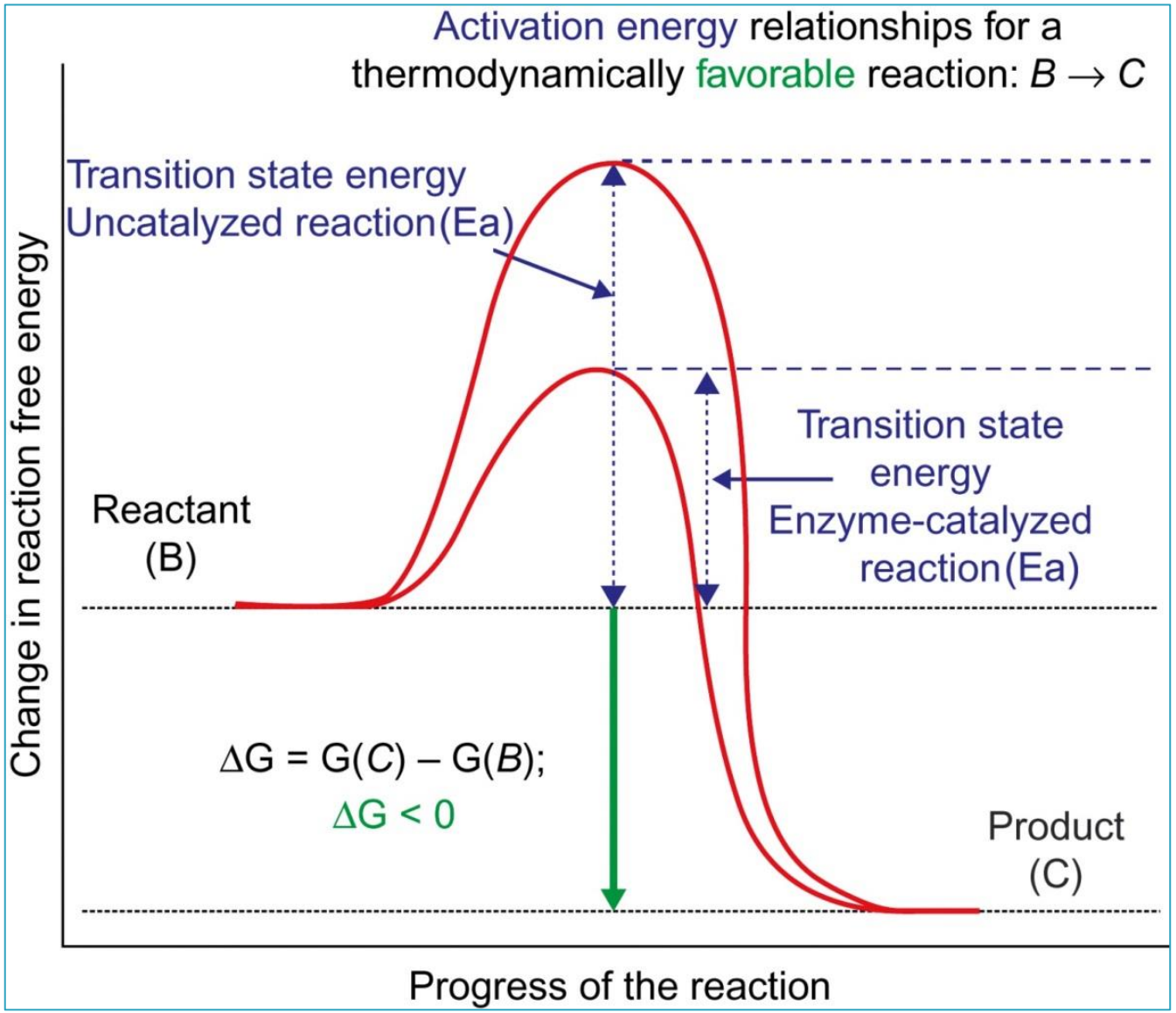
ISOENZYMES

Examples:

- Hexokinase I – IV
  - *Hexokinase IV is also called as glucokinase.*
- Lactate dehydrogenase 1 – 5
- Creatine kinase 1 – 3
  - *Creatine kinase 2 is also known as CK-MB.*

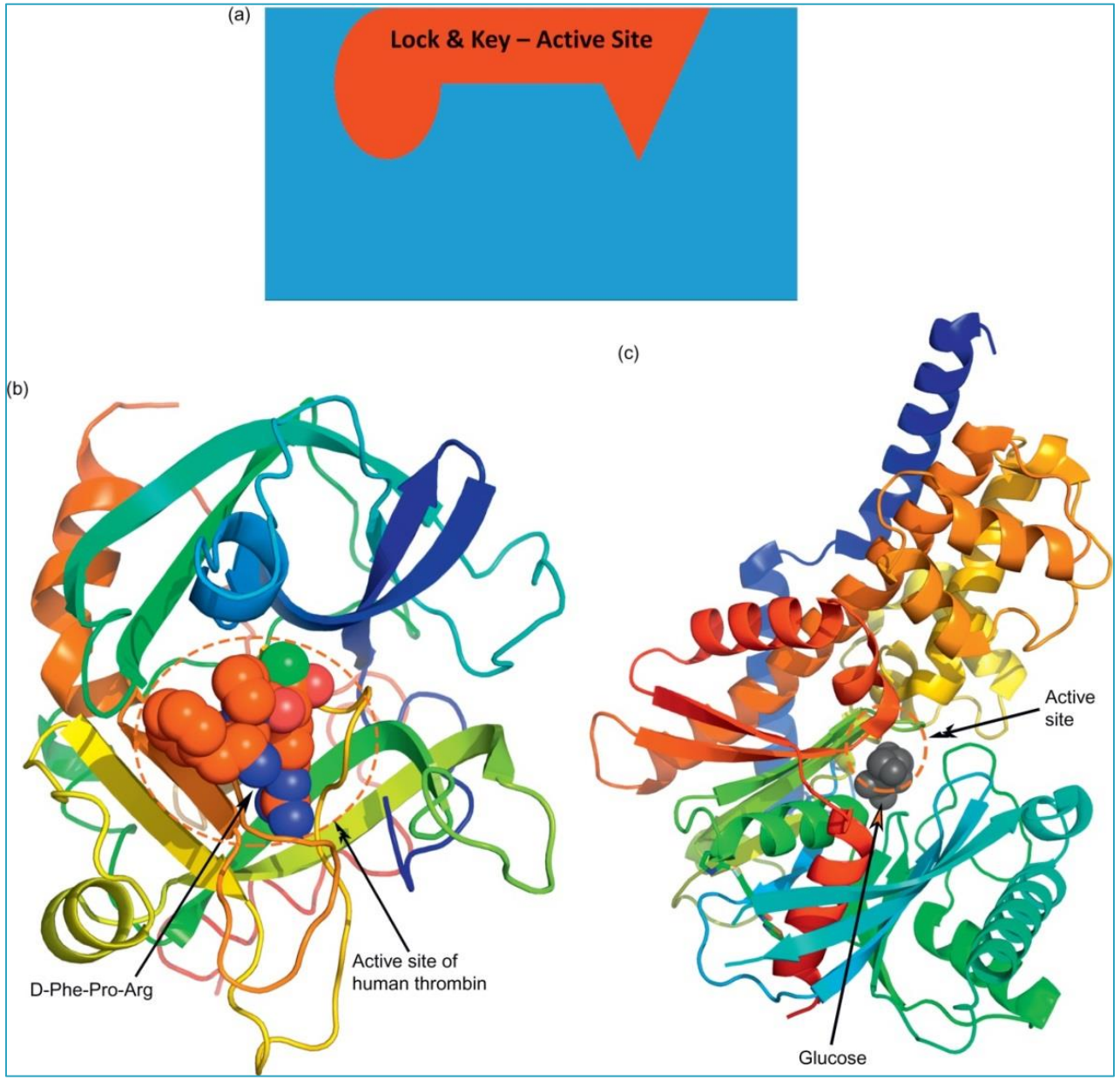
HOW DO ENZYMES WORK?

- Enzymes provide an alternate, energetically favorable reaction pathway different from the uncatalyzed reaction.
- Free energy of activation; as a result of the high free energy of activation, the rates of uncatalyzed chemical reactions are often slow.
- **The lower the free energy of activation**, the more molecules have sufficient energy to pass through the transition state, and, thus, **the faster the rate of the reaction**.
- **The enzyme** does not change the free energies of the reactants or products and, therefore, **does not change the equilibrium of the reaction**.



Chemistry of the active site

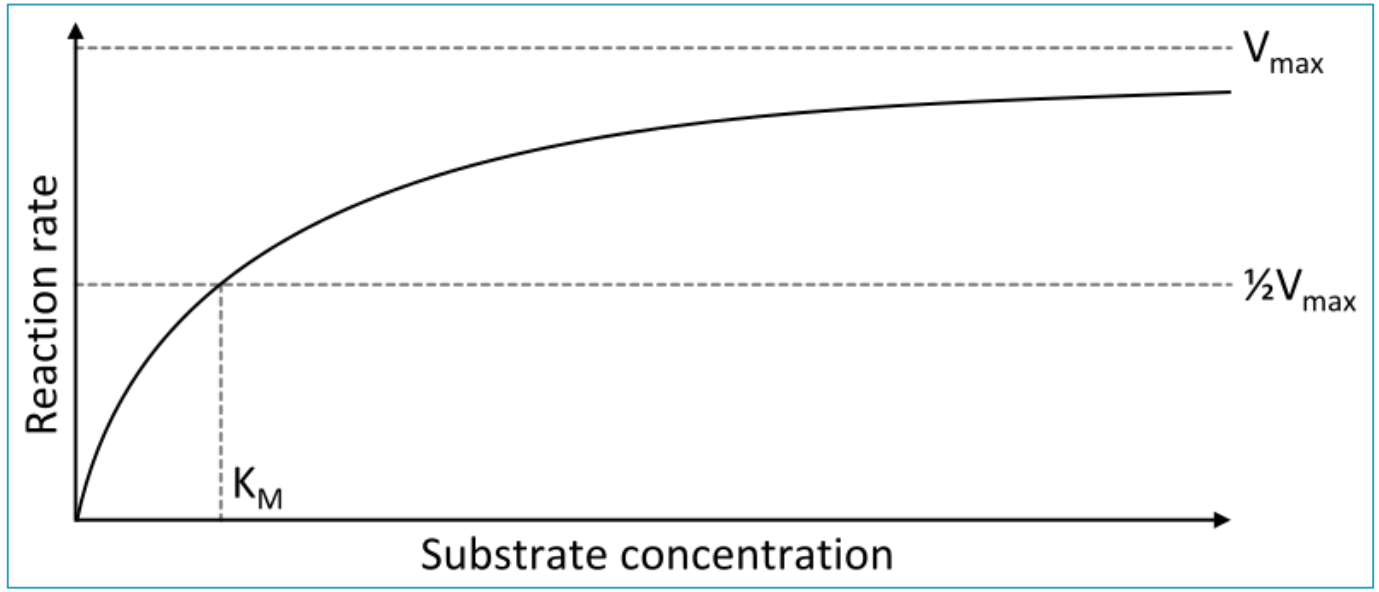
- The active site is not a passive holder for binding the substrate, but rather is a complex molecular machine employing a diversity of chemical mechanisms to facilitate the conversion of substrate to product.
  - Transition-state stabilization
    - By which, the enzyme greatly increases the concentration of the reactive intermediate that can be converted to product and, thus, accelerates the reaction.
  - The active site can provide catalytic groups
    - General acid-base catalysis
    - The transient formation of a covalent ES complex



FACTORS AFFECTING REACTION VELOCITY

- Substrate concentration
  - The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time (µmol/min; *known as international unit*, **U**).
  - The rate of an enzyme catalyzed reaction, for a given enzyme concentration, increases with substrate concentration until a maximal velocity (V_{max}) is reached.

- Most enzymes show Michaelis–Menten kinetics, in which the plot of initial reaction velocity (v_o) against substrate concentration ([S]), is hyperbolic.
- In contrast, allosteric enzymes do not follow Michaelis–Menten kinetics and show a sigmoidal curve.



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

Michaelis–Menten curve

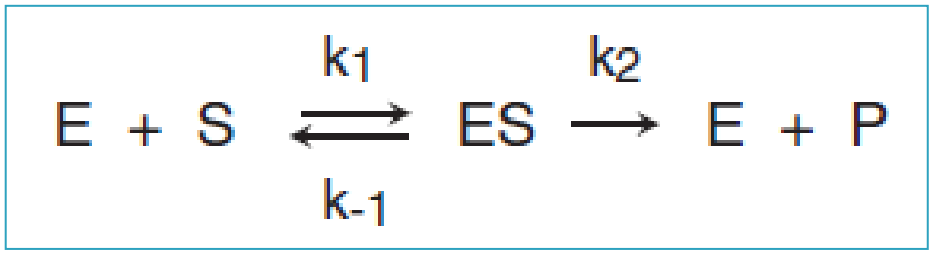
FACTORS AFFECTING REACTION VELOCITY

- Temperature
  - The reaction velocity increases with temperature until a peak velocity is reached.
  - Further elevation of the temperature results in a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme.

FACTORS AFFECTING REACTION VELOCITY

- pH
  - Effect of pH on the ionization of the active site
  - Effect of pH on enzyme denaturation
  - The pH optimum varies for different enzymes.

MICHAELIS–MENTEN EQUATION



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

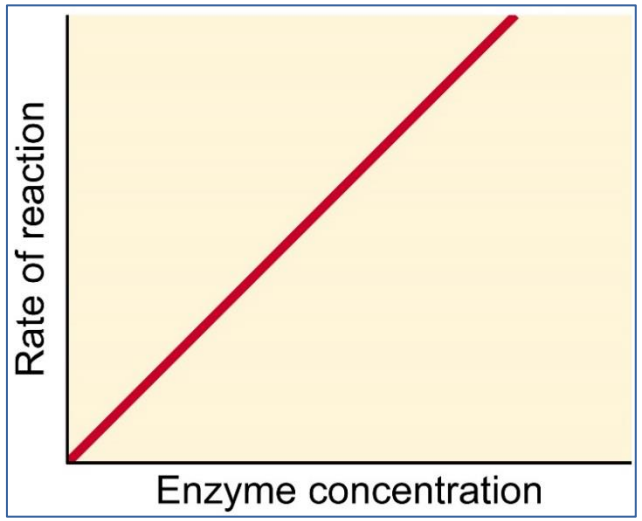
v_o = initial reaction velocity
V_{max} = maximal velocity
K_m = Michaelis constant = $(k_{-1} + k_2)/k_1$
[S] = substrate concentration

Relevant conclusions about Michaelis-Menten kinetics

- **Characteristics of Km:**
  - K_m is characteristic of an enzyme and its particular substrate, and reflects the affinity of the enzyme for that substrate.
  - K_m is numerically equal to the substrate concentration at which the reaction velocity is equal to the half of V_{max}.
  - K_m does not vary with the concentration of enzyme.
  - A numerically small (low) K_m reflects a high affinity of the enzyme for substrate, and vice versa.

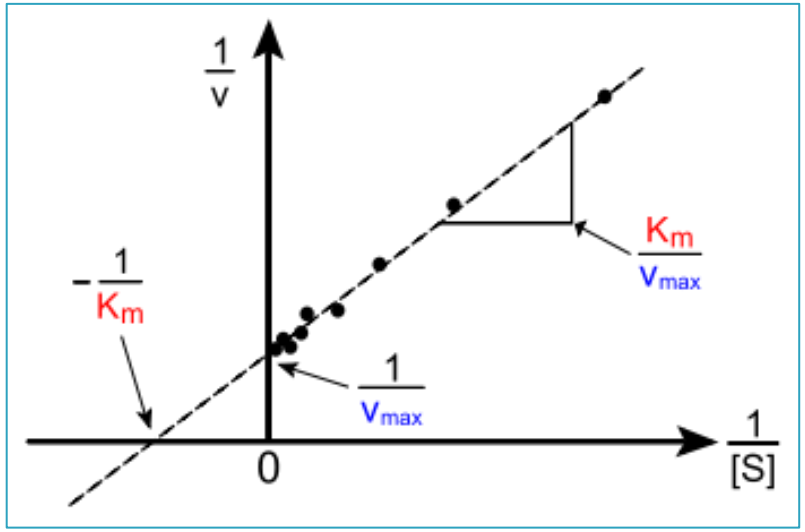
Relevant conclusions about Michaelis-Menten kinetics

- Relationship of velocity to enzyme concentration:
  - The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations.
  - For example, if the enzyme concentration is halved, the initial rate of the reaction (v_o), as well as that of V_{max}, are reduced to half that of the original.



- **Order of reaction:**
  - When [S] is much less than K_m, the velocity of the reaction is approximately proportional to the substrate concentration (first order).
  - When [S] is much greater than K_m, the velocity is constant and equal to V_{max}. The rate of reaction is then independent of substrate concentration (zero order).

Lineweaver–Burk plot

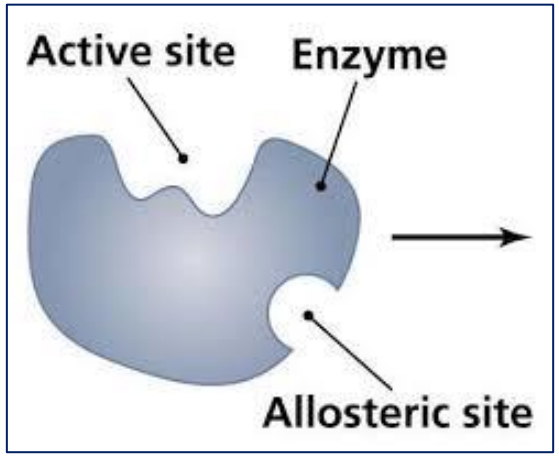


https://en.wikipedia.org/wiki/Lineweaver-Burk_plot

- The Lineweaver–Burk plot (also called a double-reciprocal plot) can be used to calculate K_m and V_{max}.
- The intercept on the x-axis is equal to $-1/K_m$, and the intercept on the y-axis is equal to $1/V_{max}$.

ALLOSTERIC ENZYMES

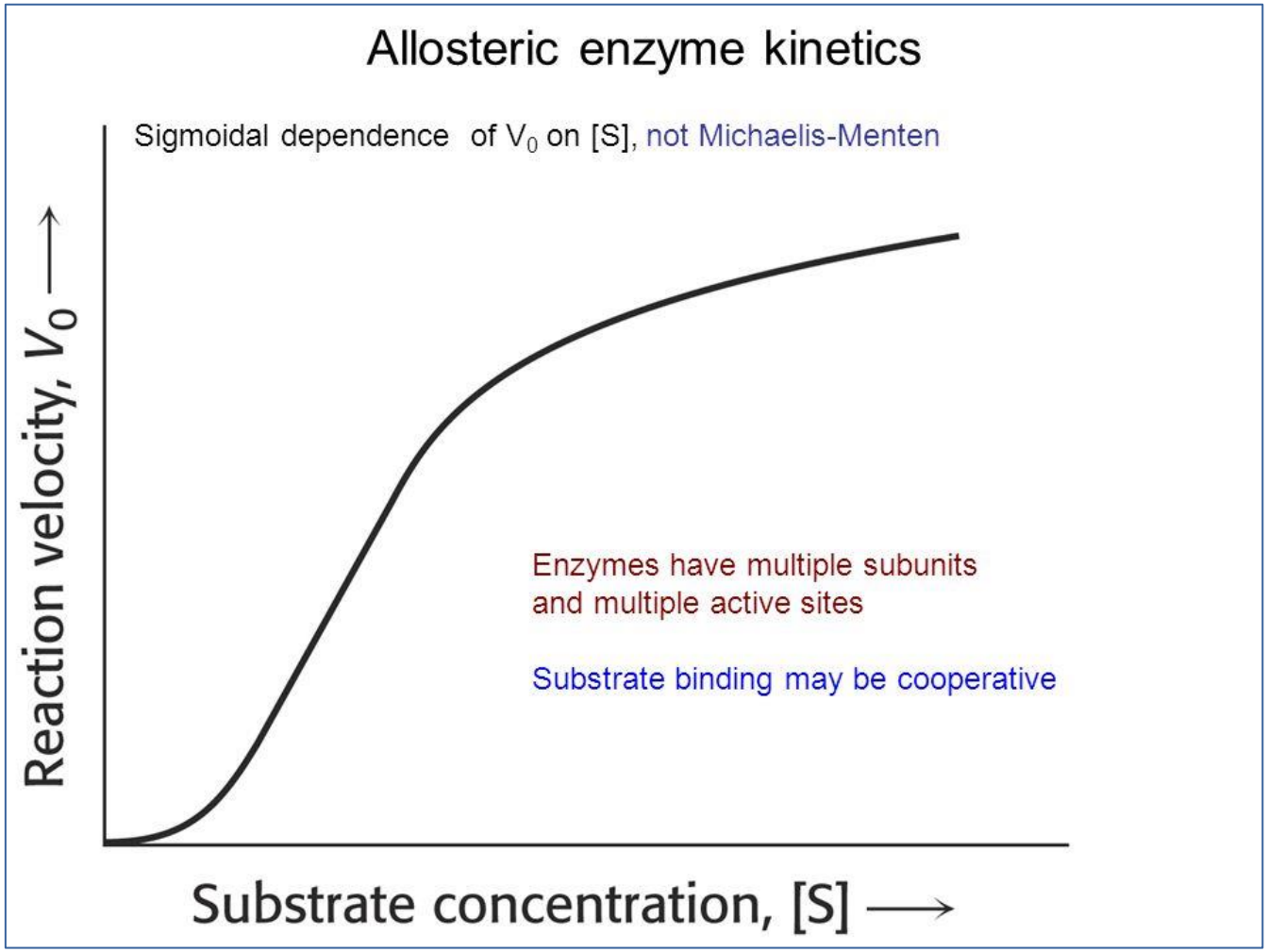
- Allosteric enzymes generally have multiple subunits.
- Allosteric enzymes are typically larger and more complex than nonallosteric enzymes.
- In addition to active sites, allosteric enzymes generally have one or more regulatory, or allosteric (besides the active site), sites for binding the modulator (effector).



ALLOSTERIC ENZYMES

- Binding of a substrate (or effector) molecule at one subunit induces a conformational change in the subunit.
- This causes a conformational changes in the other subunits.
- Allosteric enzymes show relationships between V_0 and [S] that differ from Michaelis-Menten kinetics (sigmoid curve instead of hyperbolic).
- Sigmoid kinetic behavior generally reflects cooperative interactions between multiple protein subunits.

ALLOSTERIC ENZYMES



ENZYMES IN CLINICAL DIAGNOSIS

- Plasma enzymes can be classified into two major groups.
- First, a relatively small group of enzymes are actively secreted into the blood by certain cell types. For example, the liver secretes zymogens (inactive precursors) of the enzymes involved in blood coagulation.
- Second, a large number of enzyme species are released from cells during normal cell turnover.

ENZYMES IN CLINICAL DIAGNOSIS

- These released enzymes almost always function intracellularly, and have no physiologic use in the plasma (liquid part of blood).
- In healthy individuals, the levels of these enzymes are fairly constant, and represent a steady state in which the rate of release from damaged cells into the plasma is balanced by an equal rate of removal of the enzyme protein from the plasma.
- Increased plasma levels of these enzymes may indicate tissue damage.

Change of plasma enzyme levels in disease states

- Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma.
- The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the liver, skeletal muscle, heart, and other tissues.
- The level of specific enzyme activity in the plasma frequently, **but not always**, correlates with the extent of tissue damage.
- Thus, determining the degree of elevation of a particular enzyme activity in the plasma may be useful in evaluating the prognosis for the patient.

Plasma enzymes can be used as diagnostic tools

- Some enzymes show relatively high activity in only one or a few tissues.
- The presence of increased levels of these enzymes in plasma thus reflects damage to the corresponding tissue.
- For example, the enzyme alanine aminotransferase (ALT) is abundant in the liver.
- The appearance of elevated levels of ALT in plasma signals possible damage to hepatic tissue.
- However, increases in plasma levels of enzymes with a wide tissue distribution provide a less specific indication of the site of cellular injury and limits their diagnostic value.

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