Enzymes & Coenzymes I

Lecture 7

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Editing File

Biochemistry
442

What are enzymes?

- They are **biological catalysts** that speed up the rate of a reaction **without being consumed** or changed in the reaction (speed up velocity of reaction by decreasing the amount of time needed in the reaction)

- **Structure:** all enzymes are **protein** in nature, but NOT all proteins are enzymes (some may be structural or transporters)
    - **Exception:** ribozymes are RNA (Ribonucleic acid) molecules with enzymatic activity **(441)**

- **Function:** bind to specific **substrates** (reactants) which are the substances upon which enzymes act → convert the substrates into **product(s)**

Enzyme Properties

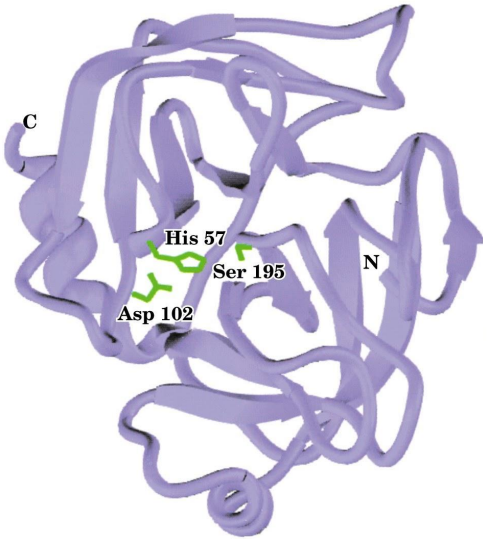
Properties:

- **Active Site:** regions where the substrate binds and where catalysis occurs (all enzymes have 1 or more active sites)

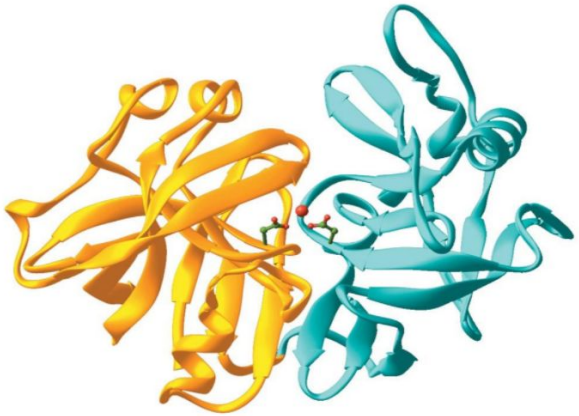
- **Specificity:** enzymes bind to **specific substrates** in the active site and are highly specific (because binding is based on the geometry/shape of substrate) → enzymes interact with **1 or few** substrates (structurally similar) and they catalyze only **1 type** of reaction even if the substance is the same **(437)**

- **Regulation:** (can be increasing or decreasing enzyme activity) enzymes can be activated (increasing) or inhibited (decreasing) so that the rate of product formation responds to the needs of the cell

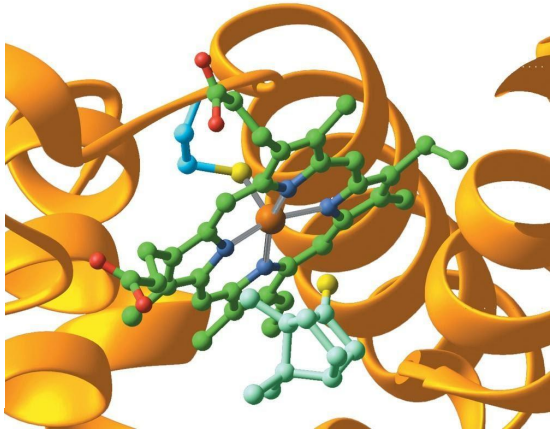
***No need to memorize structures**



Structure of Trypsin



Structure of pepsin

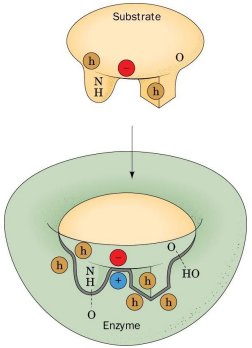


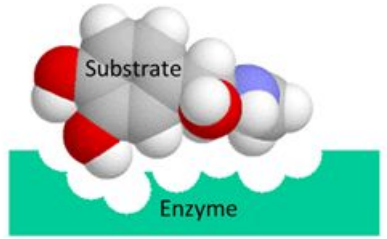
An enzyme with its active site

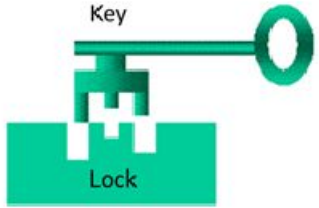
Enzyme-Substrate Binding

Two models:

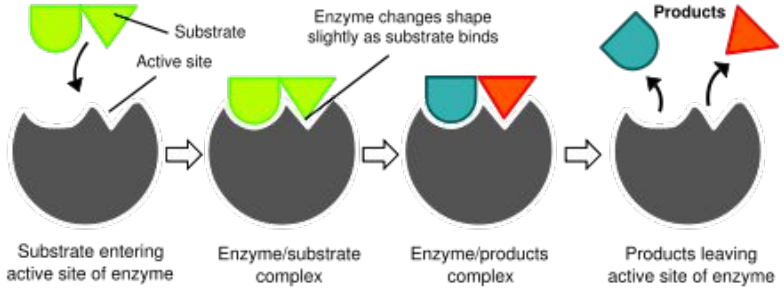
1. **Lock & Key Binding:** active site fits the **exact dimensions** of substrate (exactly complementary)



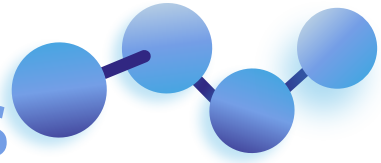




1. **Induced-Fit Binding:** after the binding of the substrate, the enzyme changes its shape or conformation to fit more perfectly with its substrate
(not fully complementary, after reaction is completed, the enzyme goes back to its original shape to function again → **441**)



Classification of Enzymes



Classified into six types according to the reaction catalyzed:

***Memorize them in order because the numbers (1 → 6) are used in systematic naming (in next slide)**

Mnemonics:

Overseas

Traveler

Heard

Lyrics

In

London

Class	Type of Reaction Catalyzed
1.Oxidoreductases	Oxidation-reduction reaction
2. Transferases	Transfer of functional groups
3. Hydrolases	Hydrolysis reactions (breaking bonds by adding water)
4. Lyases	**Group elimination** to form double/triple bonds
5. Isomerases	Isomerization (form isomers)
6. Ligases	**Bond formation** coupled with ATP hydrolysis

Enzyme Nomenclature (Naming)

	Common name	Systemic name
Rule	Suffix "**-ase**"	based on the rules given by **IUBMB** (International Union of Biochemistry & Molecular Biology): **EC Class . Subclass . Subsubclass . Enzyme number** **(EC** = Enzyme Commission**)**
Example	(E.g. amylase) Exceptions: pepsin, trypsin	Example: **EC 3.4.17.1** (carboxypeptidase A) Hydrolase, breaks a bond Which bond? peptide (439) Which enzyme exactly? hypothetically #1 Which peptide bond? alanine & serine for ex. *This is just an example, don't memorize it Just know what the numbers stand for

Cofactors & Coenzymes

- The majority of enzyme require these molecules
- They are present in the active site as an additional molecule

Holoenzymes are enzymes that require non-protein groups to catalyze a reaction (become active)

Apoenzyme
The **inactive** form of enzyme without its non-protein part

+

Non-protein part
required by some enzymes to become active; help to catalyze a reaction

=

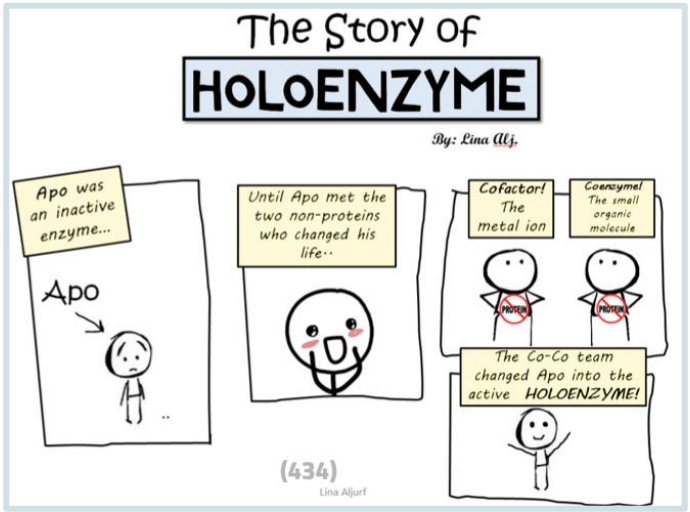
Holoenzyme
active complex
its hole is active

Coenzymes
small organic molecules,
(E.g. NAD+)

Cofactor
metal ion
(E.g. Cu2+, Fe3+, Zn2+)

Prosthetic Group
Permanently associated with an enzyme (E.g. FAD)

Cosubstrate
Temporarily associate with an enzyme (e.g. NAD)



Ribozymes

RNAs (ribonucleic acid) with enzymatic activity (they're the only non-protein enzymes)

Isoenzymes

enzymes that catalyze the **same chemical reaction** but have slightly **different structures** They're usually found in different locations [Ex. blood, tissue]

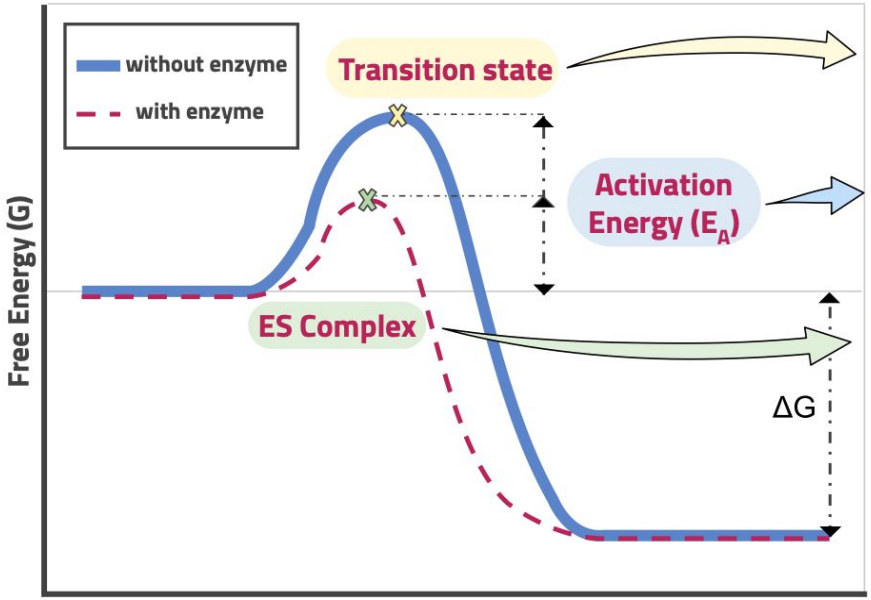
Zymogens
(Aka proenzymes)
(don't confuse with apoenzymes)

inactive enzyme precursors that require a biochemical change to become active (E.g. cleavage of a peptide blocking the active site, phosphorylation). They're activated when needed. This provides a way of regulation. For example, [pepsinogen → pepsin] only when we're eating

(434): notice that zymogens & apoenzymes need different things to become active:
Apoenzymes require a coenzyme/cofactor
Zymogens require a biochemical change

★ **Important slide**

How do enzymes work?



(441)

It is a **high-energy** intermediate that reactants pass through in every chemical reaction; it has **greater energy** that than that of reactants or products alone (the peak)

It is the **difference in energy** between **reactants** & **transition state**
- If activation energy is available, the reaction can proceed forming products
- Uncatalyzed reactions are often slow due to high E

In order for enzymes to speed up reactions, they must:
1) **decrease the activation energy** required for a reaction, providing an alternative transition state of lower energy called enzyme-substrate complex and thus speed up the reaction.
2) **NOT alter the change in the free energy (ΔG)** "energy that can do work" (441)
Why? because we don't want them to change the equilibrium of the reaction; rather, we need them to accelerate the rate by which equilibrium is reached, speeding up both forward & reverse rates

- ΔG of products must be less than ΔG of reactants
- Free energy change has to be negative for all reactions to proceed

Summary of how enzymes work:
1) Activation energy is reduced
2) Free energy remains the same

(436)
Enzyme induction = increasing activity
Enzyme inhibition = decreasing activity

Enzyme Activity or Velocity

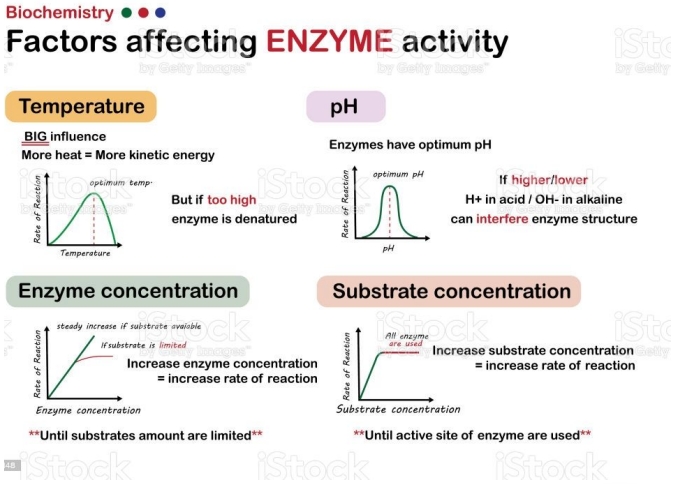
Velocity: the rate of a reaction catalyzed by an enzyme

Enzyme Activity: is expressed as **μ moles** of product formed/**min**/**mg** (per minute per milligram of enzyme) enzyme

There are 3 factors that affect enzyme activity:

- Temperature
- pH
- [S] and [E] concentration

*Square brackets [ ] depict concentration



***Note 441:**
Enzyme velocity can be measured by either:

- how much product is increased/formed (more common)
- how much substrate concentration is decreased

Factors That Affect Enzyme Activity

★ **Important slide**

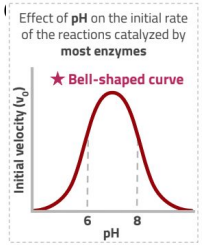
Temperature

- Every enzyme has an **optimal temp.** for catalyzing a reaction, in humans most enzymes have an optimal (enzyme is maximally active) temp of **37℃**
- The **rate** of an enzyme **initially increases** with rise in temperature (Note 441: increase in velocity until it reaches peak velocity (very active))
- But at **high** temp. (above **40℃**) enzymes are **denatured** → become **inactive**

pH

- Every enzyme has an **optimal pH** for catalyzing a reaction (peak of bell-shaped curve)
- **Most have highest activity between pH 6-8**
  (**exception:** pepsin has highest activity at **pH 2** in stomach)
- pH affects **catalysis** through either the **substrate** or **ionizable** groups in the active site of

*From Team 441 →



[S] & [E]

At **low [S]**:
- The reaction velocity/rate (v) **is proportional to [S]** (increases initially with increasing [S]

At **high [S]**:
- Further addition of **substrate** has **no effect** on enzyme activity because enzyme is saturated and all active sites are engaged
- The rate of an enzyme reaction is **directly proportional to [E]** if [S] is higher than [E]

Enzyme Kinetics

S = Substrate
E = Enzyme
ES = Enzyme-Substrate complex
P = Product
k_1, k_{-1}, k_2 = Rate constants

Note 441: In this reaction model, ES has 2 fates:
1) Continue to form product [right]
2) Go backwards [left]
(equal amounts in each direction)

Extra: Why is k_{-2} missing? Although step 2 is reversible, we do not consider its reverse reaction because our study of kinetics is during the early phase, where there is a very low concentration of product so k_{-2} is negligible

Initial Rate of Enzyme Reaction

1) Pre-steady state kinetics

When an enzyme is mixed with high [S], there is an **initial** short period of time (few 100 microseconds) during which **intermediates** leading to the formation of product **[ES] gradually build up** (no product had formed yet)

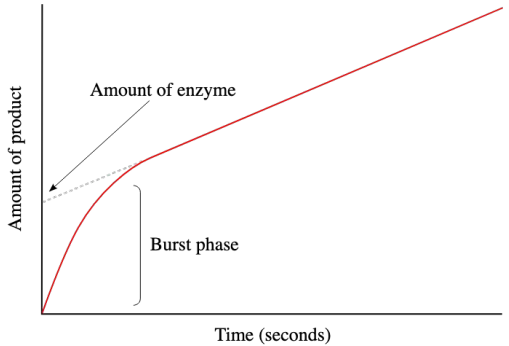
2) Steady state kinetics

After initial state, the **reaction rate** and **concentration** of intermediates change **slowly** with time and the intermediate is said to be in steady state because its rate of synthesis is equal to its rate of degradation

Rate of formation of ES = rate of breakdown of ES to (E+S) & (E+P)

Pre-steady state: ES builds up, no product yet

Steady state: ES is constant, products form



Helpful Video :)

Michaelis-Menten Equation

- The model of enzyme kinetics was first proposed by **Michaelis & Menten** in 1913 and **later modified** by Briggs & Haldane
- The **Michaelis-Menten equation** describes the relationship of **initial rate** of an enzyme to the **[S]**
- It measures the **initial velocity [V_o]** of an enzyme reaction

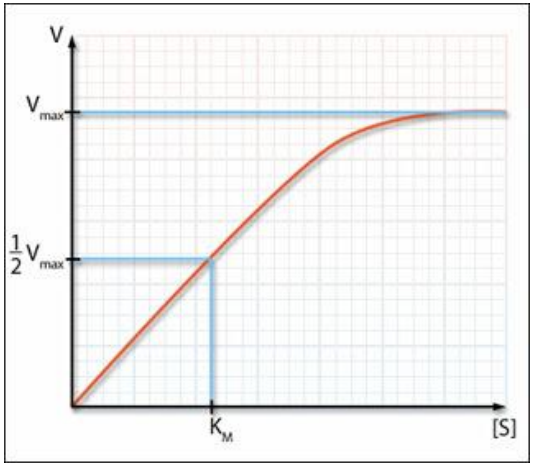
V_{max}: maximum velocity (of enzyme it *can* achieve)

K_m: Michaelis constant

[S]: substrate concentration (at which V_o is calculated)

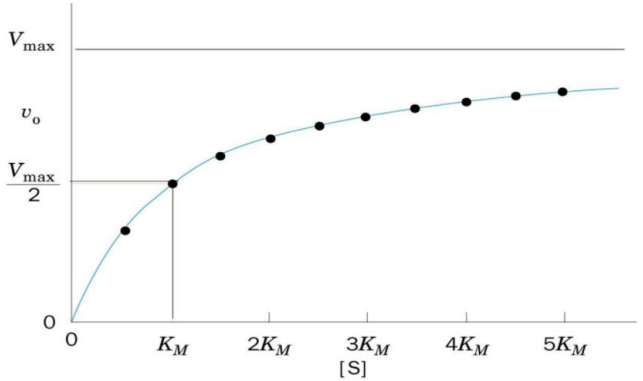
***Need to memorize equation**

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



K_m (Michaelis Constant)

- K_m is the [S] at which the **initial rate** (v_o) is one-**half** of the maximum rate (½ V_{max})
- In other words, it is the [S] required to saturate half of all of the active sites of an enzyme



Plot of the initial velocity v_o of a simple Michaelis–Menten reaction versus the substrate concentration [S]

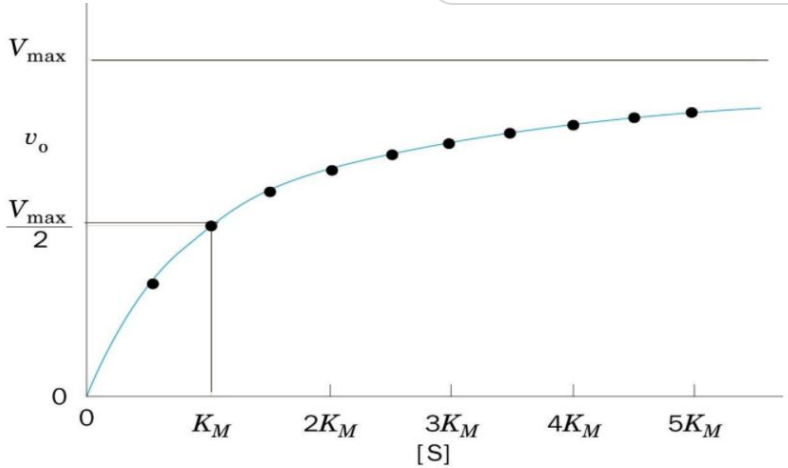
The K_m value of a substrate depends on its affinity with the enzyme:

- High K_m means low affinity with enzyme
  (more substrate needed to saturate the enzyme)
- Low K_m means high affinity with enzyme
  (less substrate needed to saturate the enzyme)

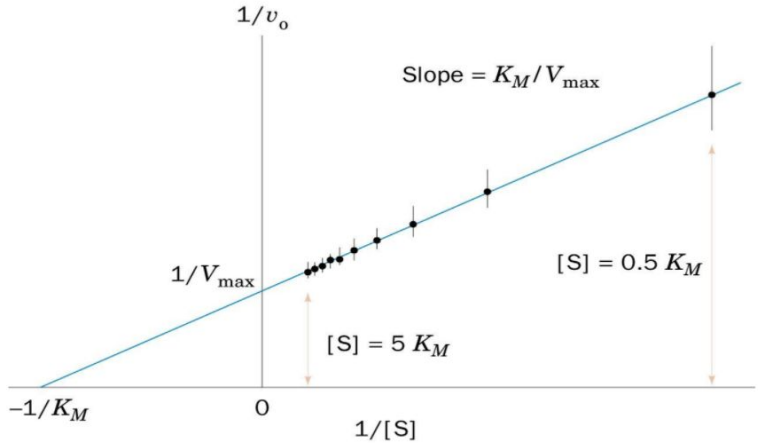
Lineweaver-Burk plot

The Lineweaver-Burk plot is a double-reciprocal plot, obtained by taking reciprocals of the Michaelis Menten equation

It is plotted to calculate the K_m and V_{max} values and to determine the mechanism of action of enzyme inhibitors



Plot of the initial velocity v_o of a simple Michaelis–Menten reaction versus the substrate concentration [S]



A double reciprocal Lineweaver–Burk plot

Quiz

Q1 : When classifying enzymes, which class functions to eliminate groups ?			
A) Oxidoreductases	B) Transferases	C) Lyases.	D) Ligases
Q2 : Some enzymes require ..... groups such as ..... to become active enzymes.			
A) Non-protein / FAD	B)Non-protein / Na	C)Binding site / Transferase	D) Binding site / Ribozymes
Q3 : Which phase contains the highest energy ?			
A) Reactants	B) Products	C) Transition	D) A and B
Q4 : K_m is the substrate concentration at which the initial rate (v_o) is:			
A) ½ of V_{max}	B) ⅓ of V_{max}	C) ¼ of V_{max}	D) ⅕ of V_{max}
Q5 : The K_m value of a substrate depends on:			
A) it's affinity with the enzyme	B) Activation energy of the reaction	C) Number of substrates involved	D) None

1. C
2. A
3. C
4. A
5. A

SAQs

Q1: Mention one difference between the pre-steady state and the steady state.

Q2: Enumerate 3 types of enzymes

SAQs Answer key:

1) Check slide 14

2) transferases, ligases, hydrolases

Our Team

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M4
E4
D2