Biochemistry of Proteins BCH 303 [Practical]

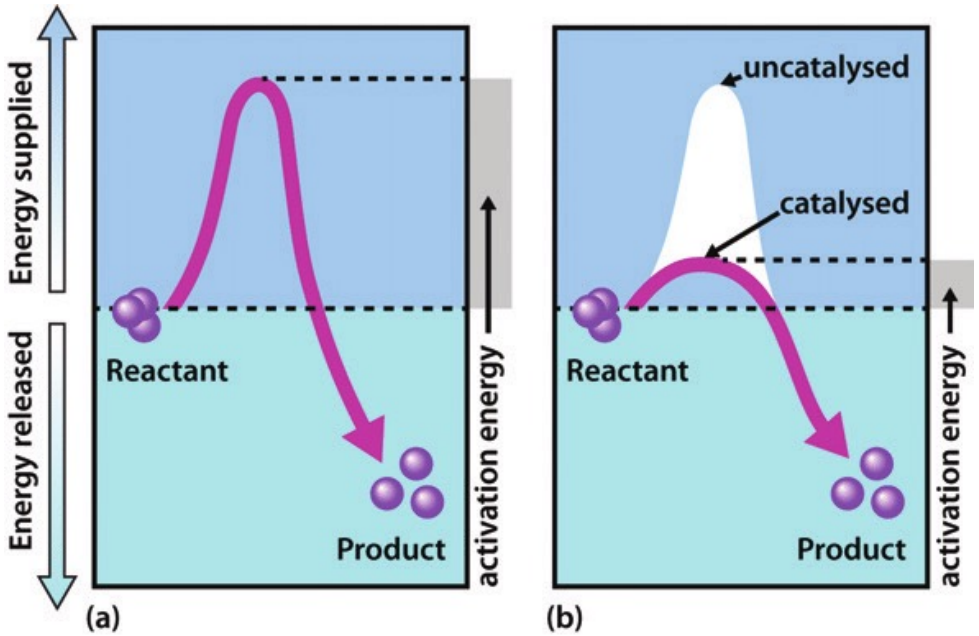
Lab (8) Effect of Various Factors on *Polyphenol Oxidase* Activity

Enzymes

- A substance that speeds up a chemical reaction—without being consumed—is called a **catalyst**
- The catalysts for biochemical reactions that occur in the living organisms are called **enzymes**
- **Enzymes** (E) are biological molecules that increase the rates [catalyze] of biochemical reactions *without being consumed.*
- Enzymes are the most remarkable and highly specialized **proteins**
- They extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts
- Enzymes have a high degree of specificity for their substrates, they accelerate chemical reactions tremendously
- They function in aqueous solutions under very mild conditions of temperature and pH. ***Why?***

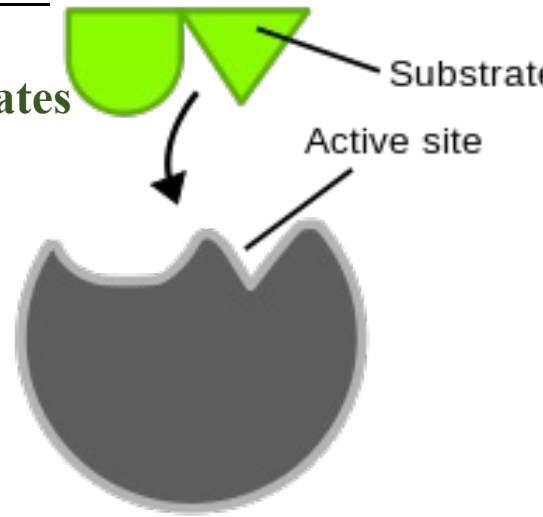
Enzymes Mechanism of Action

- **Enzymes perform the critical task of lowering a reaction's activation energy** (*that is, the amount of energy that must be supplied for a reaction to begin)*
- **Activation energy** is the energy that must be overcome in order for a chemical reaction to occur, given in units of kilojoules/ mole.



Features of Enzymatic Reactions

- **Enzymes work by binding to reactant molecules and holding them in such a way that the chemical bond-breaking and bond-forming processes take place more readily**
- The distinguishing feature of an enzyme-catalyzed reaction is that it takes place within the confines of a pocket on the enzyme called the **active site**
- **Active site** is the specific site on an enzyme where the **substrates** binds and catalysis occur
- To catalyze a reaction, an enzyme will bind to one or more reactant molecules → **substrates**
- In some reactions, one substrate is broken down into multiple products
- In others, two substrates come together to create one larger molecule



Substrate entering active site of enzyme

Enzymatic Reaction

$$\mathbf{E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P}$$

E is the enzyme

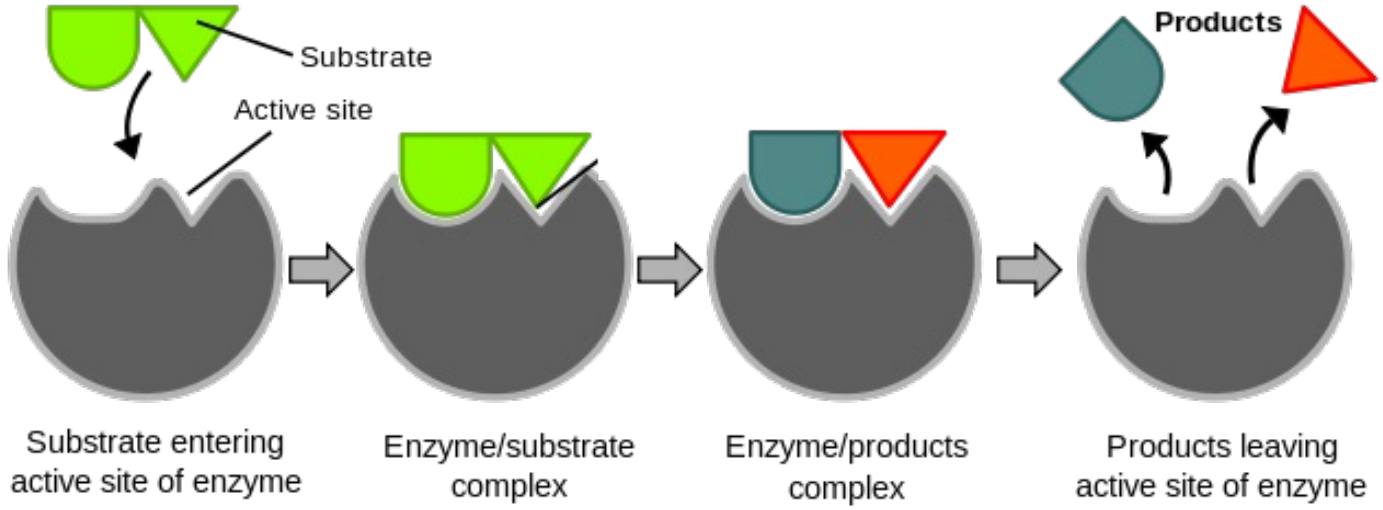
S is the substrate

P is the product

ES is the enzyme-substrate complex

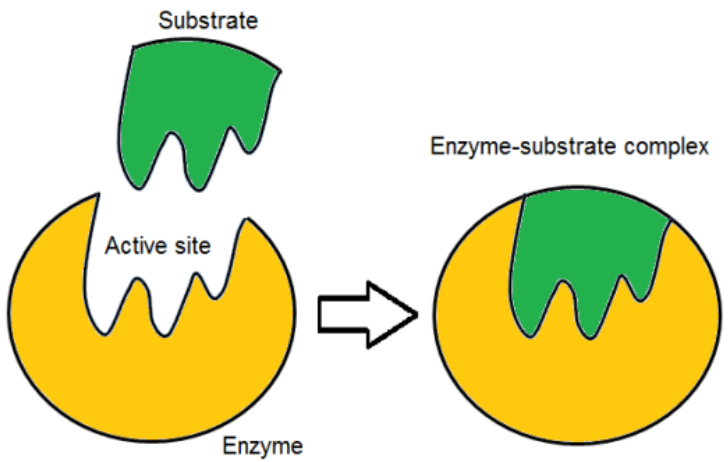
EP is the enzyme-product complex

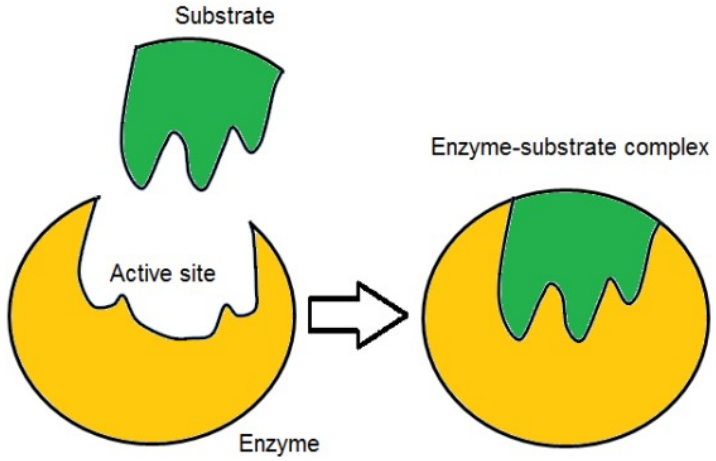
- **E-S complex** is an intermediate formed when the substrate molecule binds to the active site of the enzyme.
- **Product (P)** is a substance produced as a result of the reactions.



How can substrate bind to the enzyme?

- For many years, scientists thought that enzyme-substrate binding took place in a simple "**lock-and-key**" fashion.
- This model asserted that the enzyme and substrate fit together perfectly in one instantaneous step.
- However, current research supports a more refined view called **induced fit**. As the enzyme and substrate come together, their interaction causes a mild shift in the enzyme's structure that confirms an ideal binding arrangement between the enzyme and the substrate.
- This dynamic binding maximizes the enzyme's ability to catalyze its reaction.





Importance of studying enzyme

- Many enzymes have been named by adding the suffix "-ase" to the name of their substrate or to a word or phrase describing their activity.
- The study of enzymes has immense practical importance

→ In some diseases, especially inheritable genetic disorders, there may be a deficiency or even a total absence of one or more enzymes.

→ For other disease conditions, excessive activity of an enzyme may be the cause.

- Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in diagnosing certain illnesses.
- In addition, enzymes are important practical tools, not only in medicine but in the chemical industry, food processing, and agriculture.

Enzyme browning

- **Enzyme browning** is a usual phenomenon that can be observed commonly in fruits and vegetables, which results in quality loss of the food including the change in color, taste, flavor, and nutritional value.
- This occurs when the phenolic compounds present in them react with *polyphenol oxidase*.
- *Polyphenol oxidase (PPO)* is a copper-containing enzyme that catalyse the oxidation of dihydroxy-and trihydroxy phenol to corresponding quinone which has a brown color.
- In this lab, activity of *polyphenol oxidase* extracted from potato will be examined qualitatively

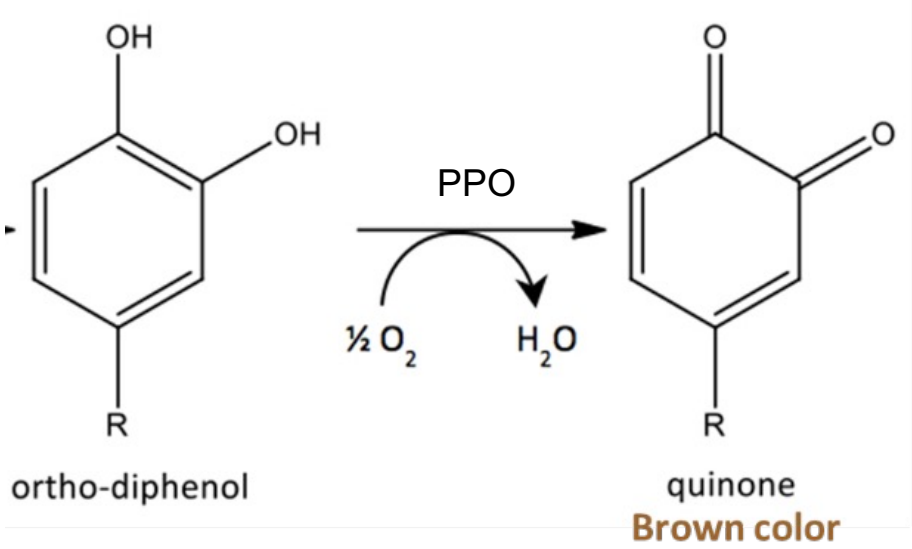


Figure 1. Oxidation-reduction reaction catalysed by polyphenol oxidase.

Practical part 🧪

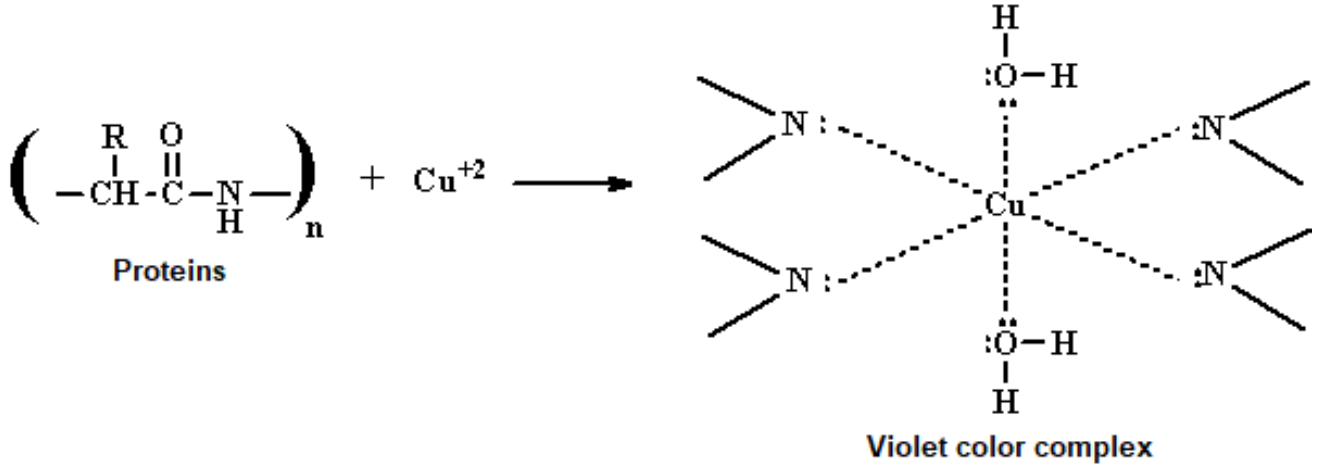
Experiment (1): Examine the protein nature of polyphenol oxidase

Aim:

- Examine the **protein nature** of polyphenol oxidase by biuret test.

Principle:

- Majority of enzymes are proteins.
- Detection of protein nature will be done using **biuret reagent**, where the peptide bonds in the proteins (enzymes) treated with an alkaline solution of dilute copper sulphate $CuSO_4$ (biuret reagent) forming a purple coloured complex.



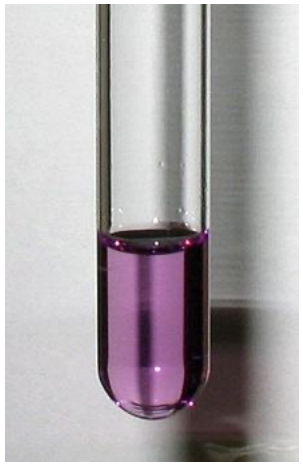


Figure 2. Biuret reaction

Experiment (1): Examine the protein nature of polyphenol oxidase

Protocol:

- Label a test tube and add 1 ml of enzyme crude extract.
- Add 2 ml of biuret reagent.

Results:

Tube	Observation
Enzyme crude extract + biuret reagent	

Experiment (2): Test the activity of polyphenol oxidase

Aim:

- To demonstrate **activity** of the enzyme.
- To investigate the **effect of incubation time** on enzyme activity.

Principle:

- Polyphenol oxidase activity will be examined qualitatively be following the change in the color.
- The oxidation-reduction reaction that catalysed by this enzyme is accompanied by a color change i.e browning (*the produced quinones spontaneously polymerize to form dark-colored phytomelanins*) (Figure 1).
- The intensity of the brown color is proportional to the enzyme's activity.

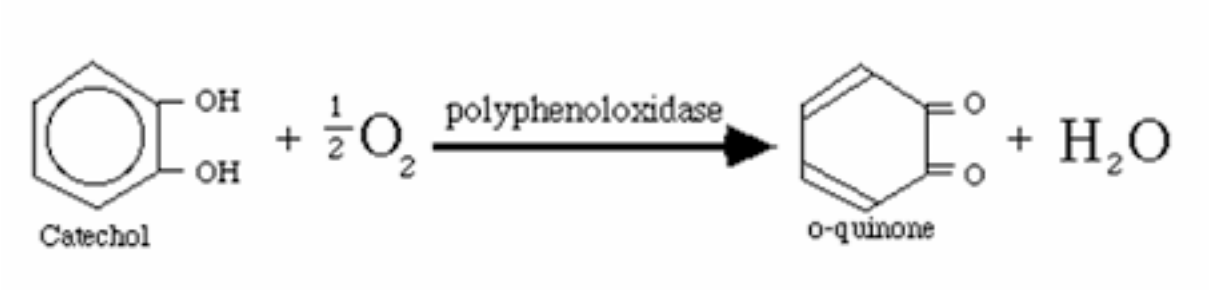


Figure 3. Oxidation-reduction reaction catalysed by polyphenol oxidase.

Experiment (2): Test the activity of polyphenol oxidase

Protocol:

- Label 3 test tube as **A**, **B** and **C**.
- In **tube A** (control): add 15 drops of the enzyme and 15 drops of catechol.
- In **tube B**: add 15 drops of the enzyme and 15 drops of distilled water.
- In **tube C**: add 15 drops of distilled water and 15 drops of catechol.
- Shake the tubes well.
- Place all the tubes in the water bath at 37 ºC. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.

Results:

Incubation time (Minutes)	Degree of color intensity (Symbol: −, +, ++ or +++)		
	A	B	C
0			
5			
10			
15			
20			
25			

Experiment (3): Demonstrate the chemical nature of polyphenol oxidase

Aim:

- To examine the **chemical nature** of *polyphenol oxidase*.

Principle:

- Polyphenol oxidase is a protein in nature, and thus effected by **various factors that affect proteins.**
- Applying an external *denaturation factors* or compound such as a strong acid or base, an organic solvent, or heat on the enzyme leading to lose its structure and so its function.
- This test depends on affecting enzyme activity as a function of changes in pH.
- In highly acidic media, the protein will be positively charged, which is attracted to the acid anions leading to protein precipitation and denaturation as a result of disrupting the salt bridges.
- In addition, enzyme activity is compromised by sequestering its cofactor.
- ***Polyphenol oxidase*** is a copper enzyme i.e. the structure of the active site of the enzyme contains two copper ions, adding of a chemical like phenylthiourea inhibit PPO by interact with copper ions at its active site.

Experiment (3): Demonstrate the chemical nature of polyphenol oxidase

Protocol:

- Label 3 test tube as **A**, **B** and **C**.
- In **tube A** (control): add 15 drops of the enzyme and 15 drops of catechol. Shake it.
- In **tube B**: add 10 drops of the enzyme and 10 drops of TCA. Shake the tube thoroughly and after 5 minutes, add 10 drops of catechol.
- In **tube C**: add 10 drops of the enzyme and few crystals of phenylthiourea. Shake the tube continually for 5 min, then add 10 drops of catechol.
- Place all the tubes in the water bath at 37 ºC for 10 minutes.
- Compare the results obtained from B and C to the control (A).

Results:

Tube	Degree of color intensity (Symbol: −, +, ++ or +++)
A (control)	
B	
C	

Experiment (4): Investigating the substrate specificity of polyphenol oxidase

Aim:

- To investigate the **substrate specificity** of the enzyme using structurally related chemicals.

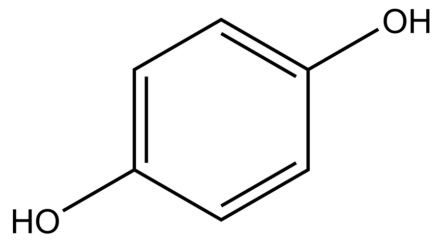
Principle:

- Enzymes bind with chemical reactants called **substrates**.
- There may be one or more substrates for each type of enzyme, depending on the particular chemical reaction.
- The enzyme's **active site** binds to the substrate.
- Since enzymes are **proteins**, this site is composed of a unique combination of amino acid residues (*side chains or R groups*). The positions, sequences, structures, and properties of these residues create a very specific chemical environment within the active site.
- A specific chemical substrate matches this site like a puzzle piece and makes the enzyme specific to its substrate.
- Potato *polyphenol oxidase* catalyses the oxidation of dihydroxy-and trihydroxy phenol to the corresponding quinine.

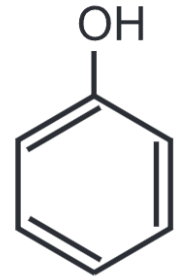
Experiment (4): Investigating the substrate specificity of polyphenol oxidase

Protocol:

- Label 3 test tube as **A**, **B** and **C**.
- In **tube A** (control): add 15 drops of the enzyme and 15 drops of catechol.
- In **tube B**: add 15 drops of the enzyme and 15 drops of phenol.
- In **tube C**: add 15 drops of the enzyme and 15 drops of hydroquinone.
- Shake the tubes well.
- Place all the tubes in the water bath at 37 ºC for 10 minutes. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.



hydroquinone



Phenol

Figure 4. hydroquinone and phenol structures

Results:

Tube	Degree of color intensity (Symbol: −, +, ++ or +++)
A (control)	
B	
C	

Experiment (5): Investigating the effect of temperature on polyphenol oxidase activity

Aim:

- To investigate the **effects of temperature** on the enzyme activity.

Principle:

- Each enzyme has a temperature that it works optimally.
- Increasing temperature above 40°C increases the rate of reaction, because it excites molecules and increases the rate at which enzymes/reactants collide and react to make product.
- However, increasing temperature too much may denature the enzyme and prevent it from working at all.
- At 0°C enzyme action is low because the movement of molecules is low.

→ This causes the collision frequency between enzyme and substrate to be low.

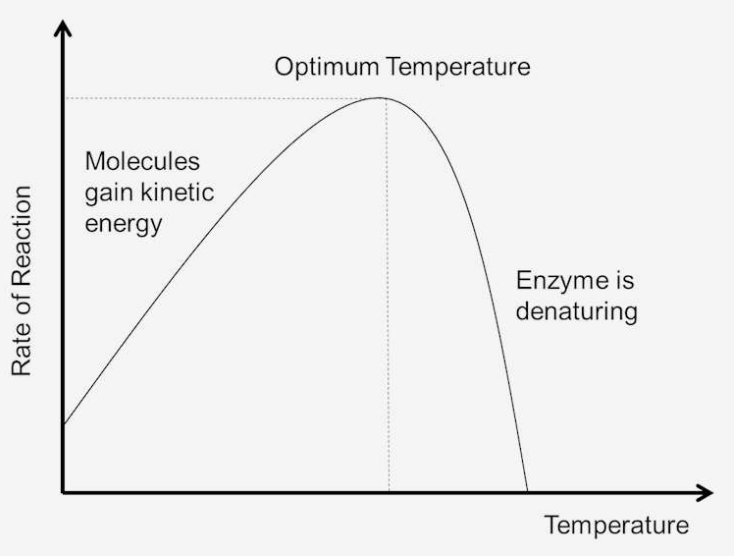


Figure 5. Effect of temperature on enzyme activity

Experiment (5): Investigating the effect of temperature on polyphenol oxidase activity

Protocol:

- Label 3 test tube as **A**, **B** and **C**.
- In **tube A**: add 15 drops of the enzyme and incubate at 0 ºC for 10 min.
- In **tube B**: add 15 drops of the enzyme and incubate at 37 ºC for 10 min.
- In **tube C**: add 15 drops of the enzyme and incubate at 95 ºC for 10 min.
- Add 15 drops of catechol for all tubes.
- Shake the tubes well, the return the tubes to the proper temperature.
- Wait for 15 minutes. Then, examine each tube without removing it from its temperature condition

Results:

Temperature (ºC)	Degree of color intensity (Symbol: −, +, ++ or +++)
0	
37	
95	

Homework

- Search for an application of enzymes in the industry and describe it briefly.