Enzymes - Exercise 3 - Germantown

Objectives

-Understand the function of an enzyme.
-Know where catechol oxidase (enzyme) used in today's experiment came from.
-Understand why enzymes require a cofactor.
-Understand how PTU (phenylthiourea) affects the enzyme.
-Understand the specificity for enzymes affect the molecules Catechol & Hydroquinone
-Understand how at different environments (temperature & pH affect an enzyme.

- Enzymes are proteins that act as catalysts. Catalysts increase the rate of a chemical reaction without being consume by the reaction. Enzymes are not altered during a reaction and therefore they can be reused again and again.

- An enzyme speeds up a reaction by lowing the reaction's activation energy. All reactions require some energy to get started (activation energy). Enzymes lower the activation energy they do not provide activation energy. All chemical reactions require activation energy (Ea). Without enzymes reaction in cells would be slow and take a very long time.

- Enzymes are specific for certain reactions. There three dimensional structure (tertiary structure) determines their specificity (FORM FITS FUNCTION). Temperature, pH, and salt concentration can alter the enzyme's tertiary structure, thereby alerting the enzyme's function.

- In an enzymatic reaction, substrates bind to an enzyme's active site, creating an enzyme-substrate complex (ES). The enzyme-substrate complex then turns into an enzyme-product complex (EP). Finally, the enzymes detach from the product (P) and the reaction is complete.

- The function of the enzyme is dependent on the shape of its active site.

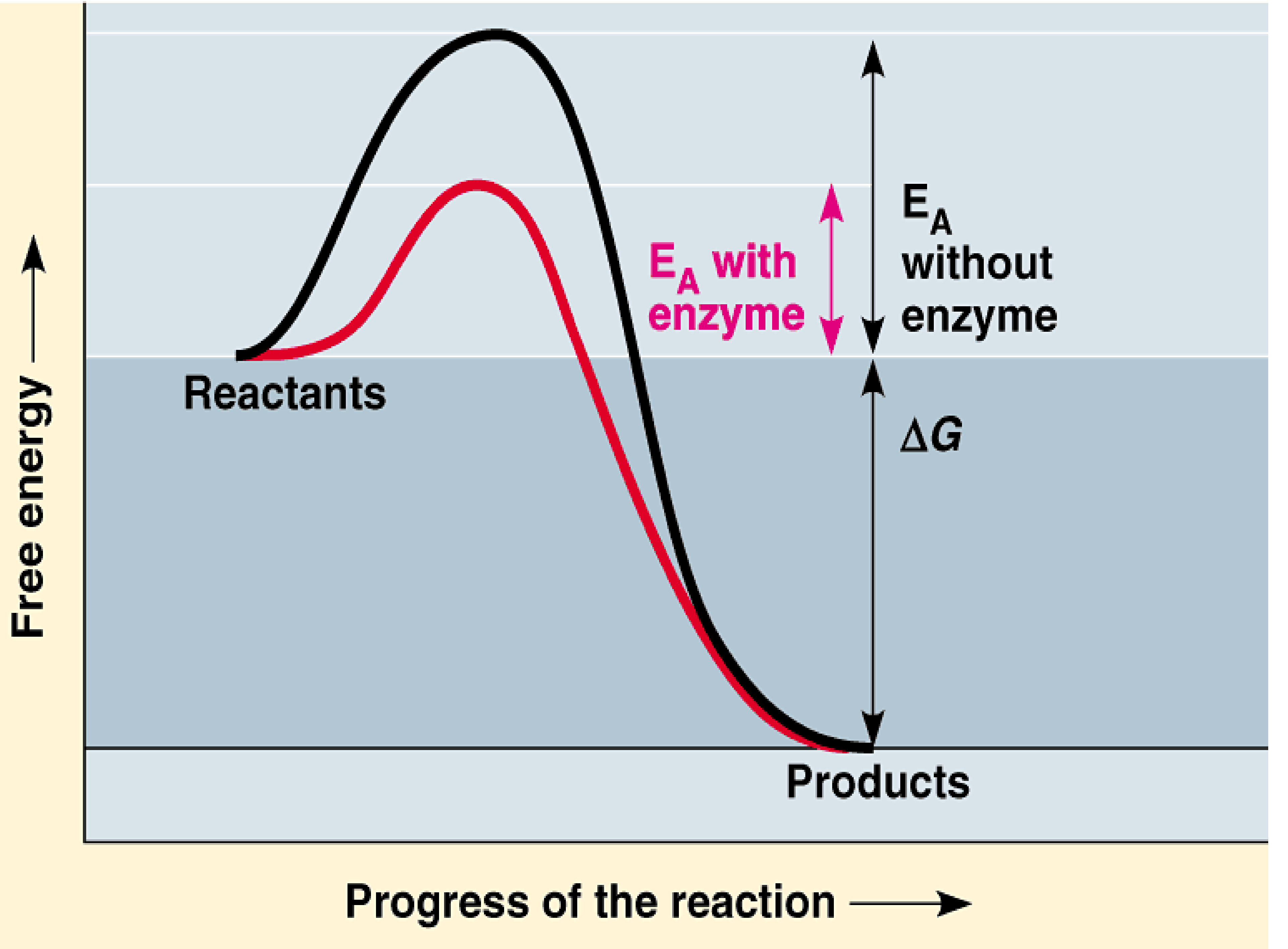
Enzymes

- Speed up chemical reactions (catalysts).
- Work by lowering the activation energy.
- Reactants the enzyme acts upon are called substrates.

sucrose + sucrase + H_2O → glucose + fructose

(substrate) (enzyme) (water) (products)

- Very specific for reactions.
- Three dimensional shape determines function (tertiary, quaternary structure).
- Active site is region where the substrate binds.
- Enzymes are not altered in a reaction and can be used again.


Free energy
Reactants
E_A with enzyme
E_A without enzyme
ΔG
Products
Progress of the reaction

Substrate Specificity of Enzymes

- The substrate
  - Is the reactant an enzyme acts on
- The enzyme
  - Binds to its substrate, forming an enzyme-substrate complex
- The active site
  - Is the region on the enzyme where the substrate binds

In an enzymatic reaction the substrate binds to the active site

- The catalytic cycle of an enzyme

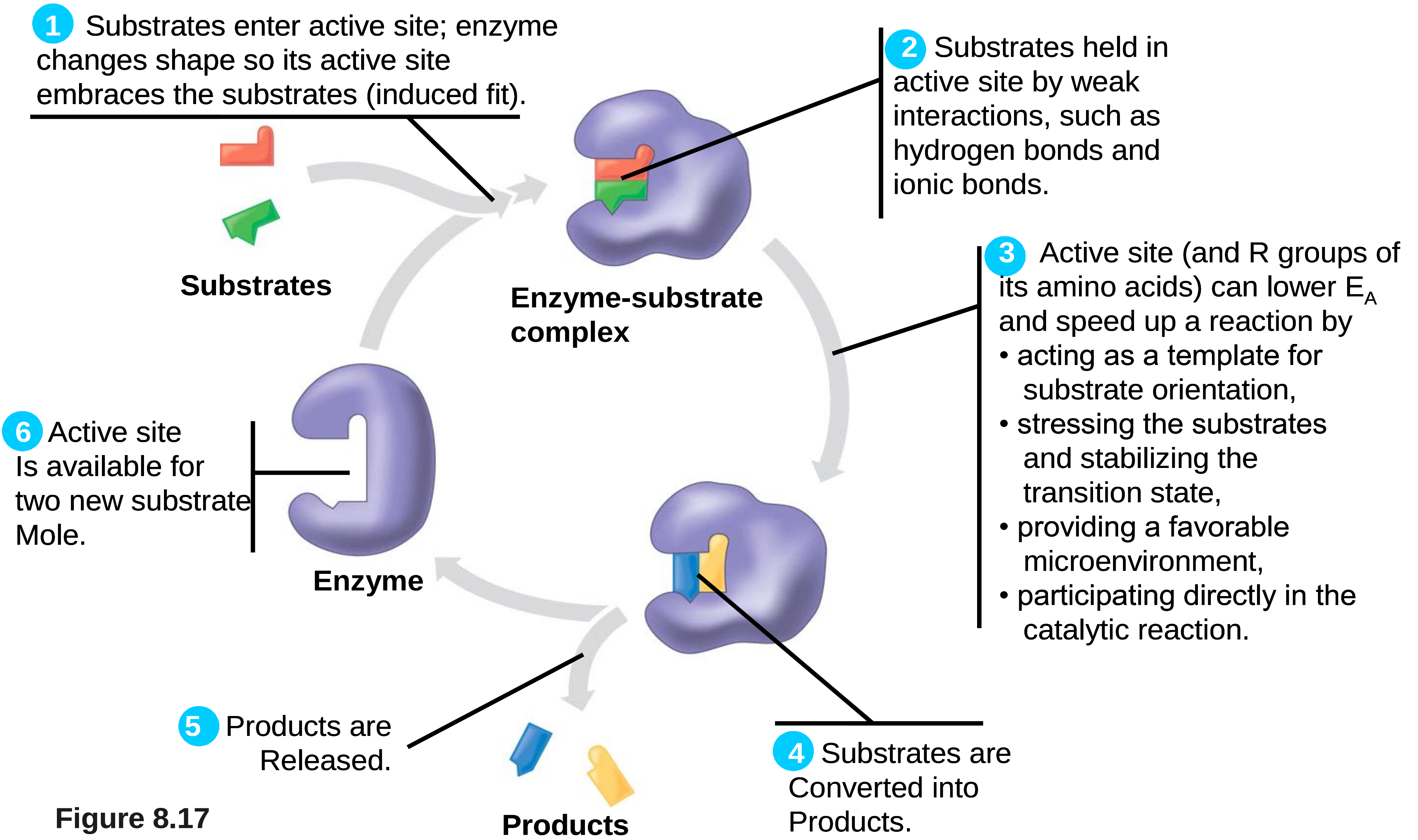


Figure 8.17

When plant tissue is exposed to the air, it produces a brown colored chemical called benzoquinone. Benzoquinone accumulates in the tissue and helps it to protect it from microbes , such as, bacteria and fungi that cause riot. This protective reaction in plants is shown here:

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catechol + oxygen gas --------------------→ benzoquinone + water
catechol oxidase

In all of these cases, oxygen gas in the air is combined with catechol in the damaged plants cells to produce benzoquinone

Three notable examples of this reaction in plants is observed in a peeled potato exposed to air, in a partially eaten apple, or in a pear or other fruit that has been bruised.

The enzyme catechol oxidase to be used. It was derived from fresh potatoes made into a milky mixture called potato extract and stored on ice in brown bottles.

The potato extract contains the enzyme catechol oxidase.

So how do you know when there's a reaction? When the reaction turns a brownish color that tells us the activity of the enzyme. The darker brown it is, the more product you have for that particular environment, wheatear it is temperature or pH.

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Detecting the Product of the Reaction

Since the substrate (catechol) is nearly colorless, and the product (benzoquinone) is brownish in color, we can use a color scale to monitor the progress of the reaction and the activity of the enzyme in a test tube environment. Once we become familiar with the color of the test tube when the reaction is completed, and the color of the tube when the reaction fails to occur, we can use those colors as a basis or standard to compare to other tubes in later experiments. The color scale we will use is as follows:

Color	Progress of the reaction	Indicator scale (from 0 to +4)
milky, clear	no reaction	0
murky, less clear	reaction is just beginning	+1
beige to light brown	some of the product is formed	+2
medium brown	most of the product is formed	+3
amber to dark brown	product is formed; reaction is completed	+4

catechol + oxygen gas --------------------→ benzoquinone + water
catechol oxidase

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Tube	Chemicals to add
E	3ml potato extract; 3ml 1% catechol
C1	3 ml potato extract; 3 ml distilled water
C2	3 ml 1% catechol; 3 ml distilled water

- 2. What two chemicals are necessary produce the brown product?
  Catechol & oxygen (oxygen gas)

- 3. Why are tubes C1 and C2 called control tubes?

C1) Has 3ml potato extract; 3 ml distilled water

C2) Has 3 ml 1% catechol; 3 ml distilled water, it has both of the chemicals that we tested but in the experiment we put both substances

- Many enzymes require a cofactor (metal ion or coenzyme), which acts to either maintain the shape of the enzyme molecules or to interact with the substrate at the active site of the reaction can proceed. Another example is Fe with RBC's. Fe carries the oxygen.
- **Cu is the cofactor for catechol oxidase.**
- PTU is an abbreviation for the molecule phenylthiourea, which can bind to copper ions (Cu), cofactor, to the enzyme.

PTU - Phenylthiourea

- PTU: This would bind to Cu+, so if there's only Cu in the solution of enzyme the PTU is going to bind and pick up the Cu.

- What you're testing is whether the enzyme needs Cu, so tested this with and without PTU too. Not testing whether PTU is cofactor, the test is to see whether Cu is cofactor, the PTU is strictly used to make Cu out of the solution. **Cu is known as the cofactor.**

- **PTU is a chemical that binds Cu better than the enzyme binds Cu. So what it does is it goes around, and it acts like a vacuum cleaner, and takes all the Cu from any of the enzymes that has it.**

- **If this enzyme Catechol oxidase needed Cu, Cu is no longer available when PTU is there because the PTU got the Cu because it likes it better than the Catechol oxidase (the enzyme).**

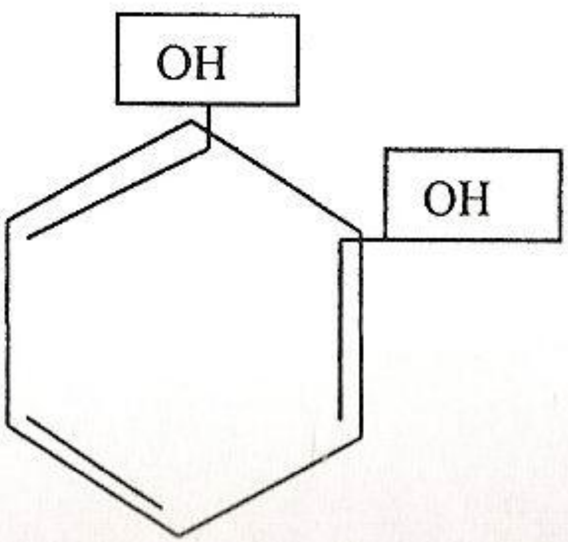
- **The Cu got taken away, so the enzyme doesn't work if it doesn't work Cu. So somebody stole its Cu. So because it was inhibited it does require Cofactor because it was inhibited.**

- **Adding PTU makes the enzyme not function.**

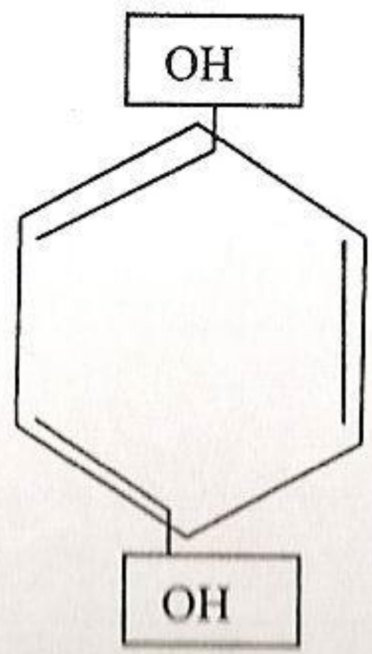
Specificity for enzymes - Page 34 – Lab Book

Notice that the position of the hydroxyl (OH-) groups is different in the two molecules. For catechol, the hydroxyl groups to the benzene ring are adjacent to one another, while for hydroquinone the hydroxyl groups are opposite one another. In other words, they have each a slightly different shape.

Catechol



Hydroquinone



Specificity for Enzymes - **Catechol & Hydroquinone**

- If the temperature and pH are optimal, the enzyme will bind tightly to the substrate and the chemical reaction will occur. In this section of the lab exercise, you will compare two very similar, though not identical, molecules and determine if either one of them or both of them can be hydrolyzed by the enzyme oxidase into the brown product benzoquinone:

- 2 molecules: Catechol and hydroquinone. They have slightly different shapes.

- Procedure:

-To tube A, add 3 ml of potato extract and 3 ml of 1% catechol.

-To tube B, add 3 ml of potato extract and 3 ml of 1% hydroquinone.

Effect of Temperature on the rate of Enzyme activity

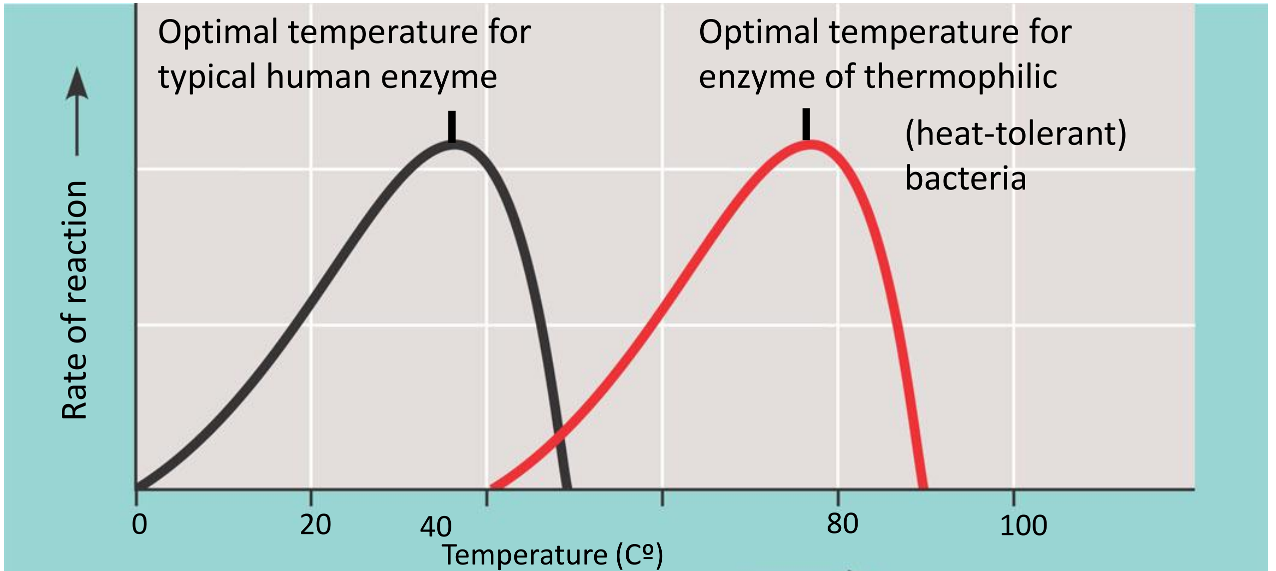
- Chemists have long known that an increase in temperature will increase the rate of a chemical reaction. Effect of temperature on the activity of the enzyme catechol oxidase.

- Consider the following two facts: **1) An increase in temperature increases molecule motion and therefore collision rates between substrates and enzyme. 2) Enzymes, being globular proteins, are denatured at high temperatures. Their shape is changed as the high temperature breaks some chemical bonds that maintain secondary and tertiary protein structure. In this way, the shape of the enzymes active site is altered.**

- Temperature: Optimal: Effect of temperature of enzyme activity. Proteins are denatured at high temperatures.

Effects of Temperature

- Each enzyme
  - Has an optimal temperature in which it can function



(a) Optimal temperature for two enzymes

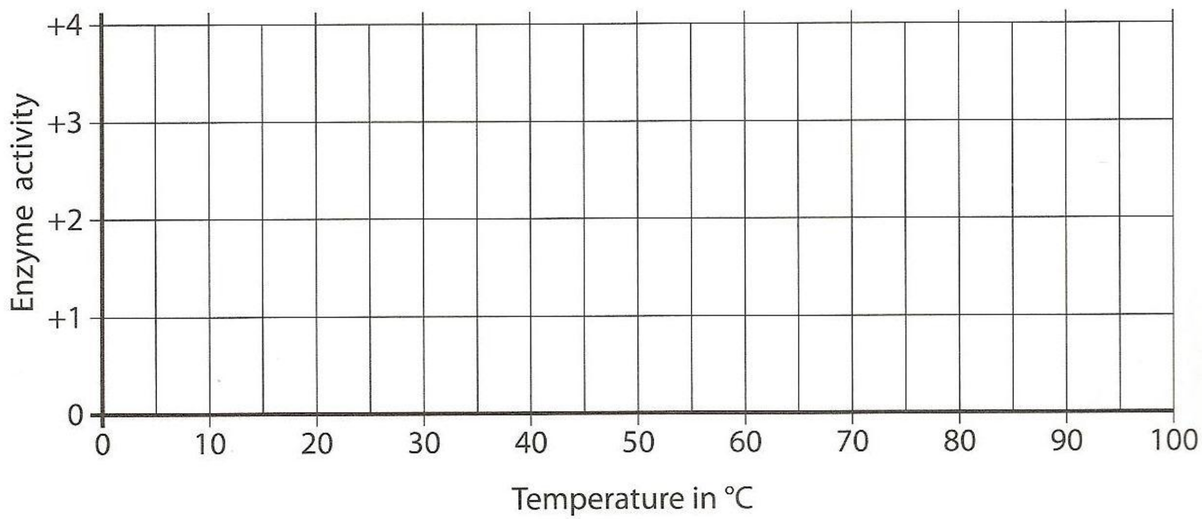
Figure 8.18

Temperature **Page 37 – Lab Book**

Table 3.4

Tube	Environmental temperature (°C)	Color at 0 minutes	Color at 5 minutes	Color at 10 minutes
5	ice at 5°			
22	22° room temp.			
37	37° water bath			
C (control)	37° water bath			
60	60° water bath			
80	80° water bath			
100	boiling at 100°			

The Effect of Temperature on the Rate of the Enzymatic Reaction

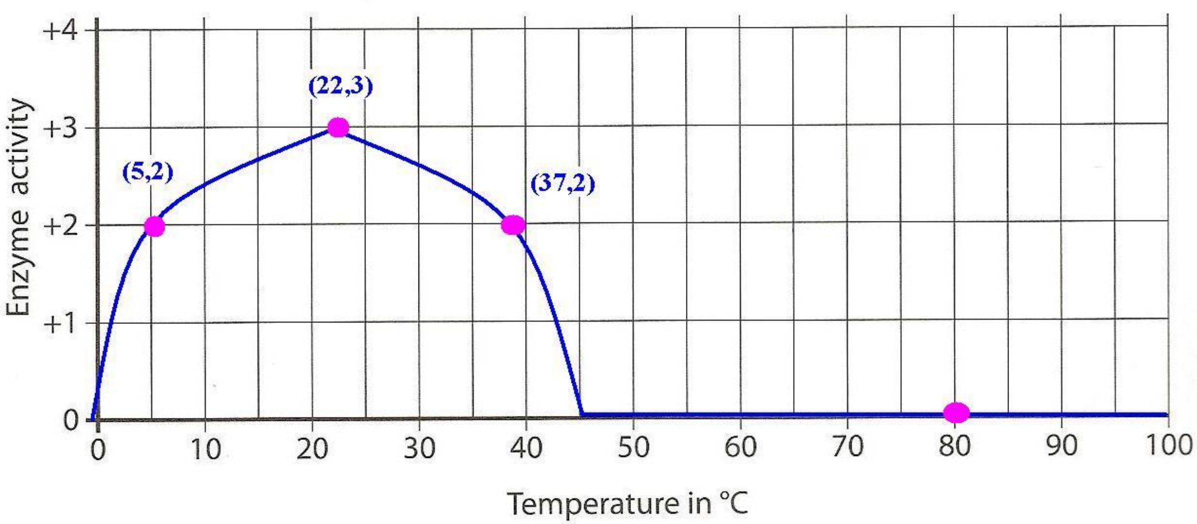


Temperature **Page 37 – Lab Book**

Table 3.4

Tube	Environmental temperature (°C)	Color at 0 minutes	Color at 5 minutes	Color at 10 minutes
5	ice at 5°	1	3	2
22	22° room temp.	2	3	3
37	37° water bath	2	3	2
C (control)	37° water bath	0	0	0
60	60° water bath	0	0	0
80	80° water bath	0	0	0
100	boiling at 100°	0	0	0

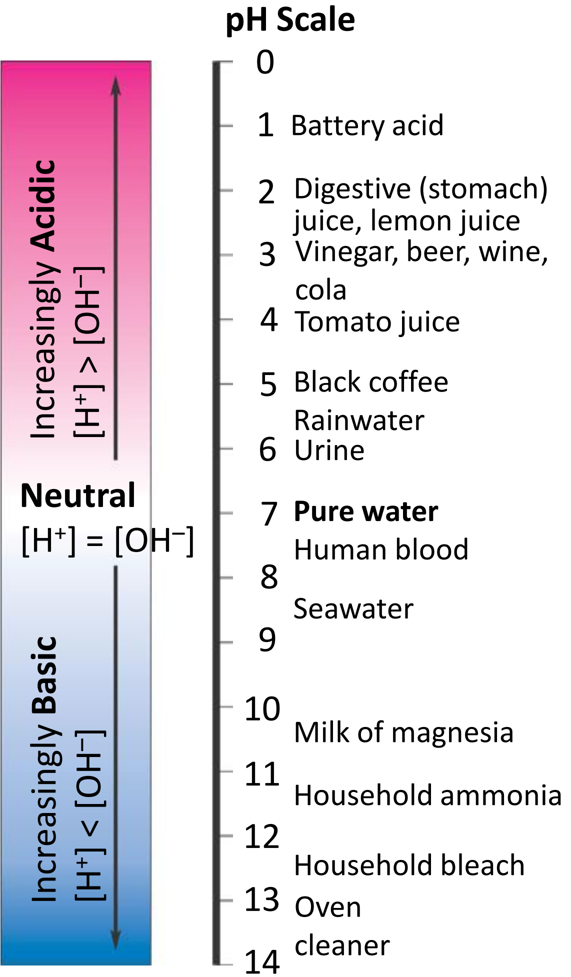
The Effect of Temperature on the Rate of the Enzymatic Reaction



Effect of pH on the Rate of Enzyme Activity

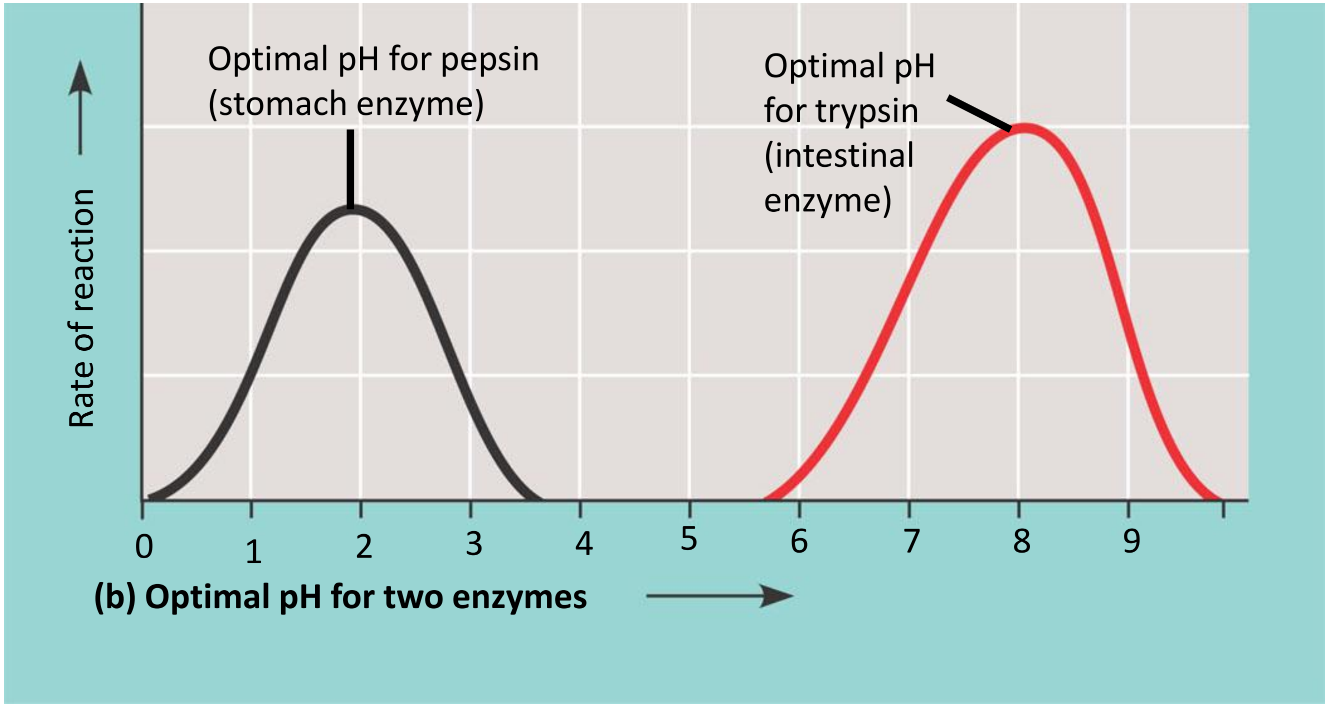
- **All enzymes have a pH optimum at which they are able to catalyze reactions most efficiently**. To analyze the effect of different pHs on the activity of the enzyme catechol oxidase, the use of buffers is required. **Buffers are substances that help maintain a constant pH in a mixture by stabilizing the amount of hydrogen ions present in the mixture. The experiment will use buffers that vary from strong acids (pH 2) to neutral (pH 7) to strong bases (pH 12).** **Enzymes, proteins, are practically or completely denatured if the pH is changed from the optimum pH.**

Enzymes have an optimal pH in which it can function



The pH of a solution

- Is determined by the relative concentration of hydrogen ions (H+)
- Is low in an acid
- Is high in a base

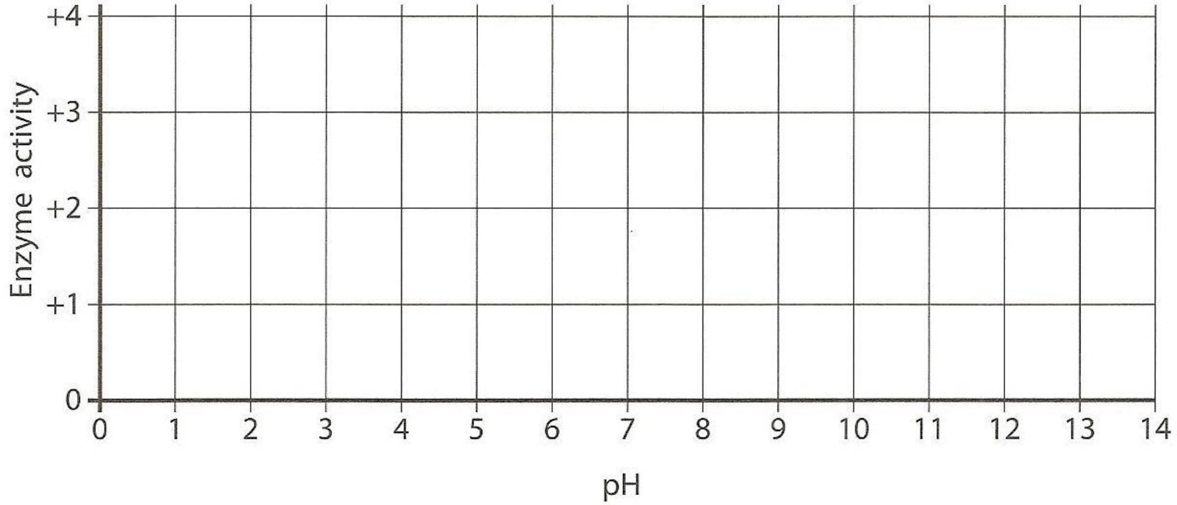


pH **Page 40 - Lab Book**

Table 3.5

Tube	pH of tube	Color at 0 minutes	Color at 5 minutes	Color at 10 minutes	Color at 15 minutes
2	2				
4	4				
6	6				
7	7				
7C (control)	7				
8	8				
10	10				
12	12				

The Effect of pH on the Rate of the Enzymatic Reaction

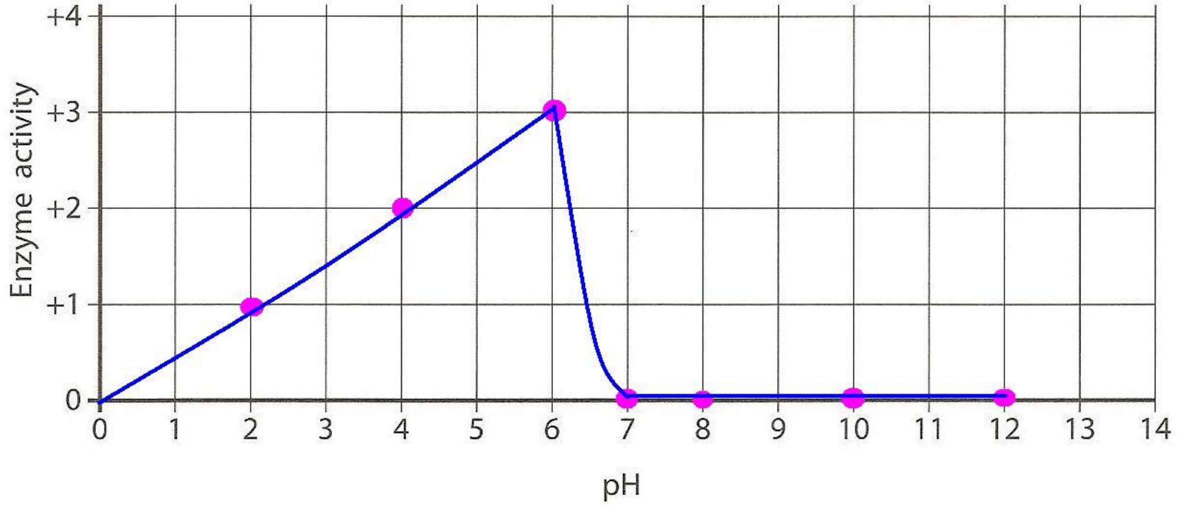


pH **Page 40 - Lab Book**

Table 3.5

Tube	pH of tube	Color at 0 minutes	Color at 5 minutes	Color at 10 minutes	Color at 15 minutes
2	2	1	1	1	1
4	4	0	2	2	2
6	6	3	3	3	3
7	7	3	0	0	0
7C (control)	7	0	0	0	0
8	8	3	0	0	0
10	10	2	0	0	0
12	12	0	0	0	0

The Effect of pH on the Rate of the Enzymatic Reaction



Questions

- 1. What is an Enzyme?

- 2. Enzymes increase the rate of a reaction by.

A. Decreasing the requirement of activation energy.
B. Providing the activation energy to the reaction.
C. Both A & B
D. None of the above.

- 3. Do you expect an enzyme to be active at 0 degrees C. Why?

- 4. Name three physical and chemical factors that affect enzyme's activity (denature a protein/enzyme)?

- 5. All enzymes function optimally at PH 7.4
  True or False

Questions

- 1. What is an Enzyme: **A protein catalyst that speeds up a reaction without getting consume in a chemical reaction. It lowers the activation energy barrier too.**

- 2. Enzymes increase the rate of a reaction by.

A. Decreasing the requirement of activation energy.

B. Providing the activation energy to the reaction.

C. Both A & B

D. None of the above.

- 3. Do you expect an enzyme to be active at 0 degrees C. Why? **Yes, the enzyme is still active but is working at slower rates.**

- 4. Name three physical and chemical factors that affect enzyme's activity (denature a protein/enzyme)?

  Temperature, pH, Concentration (salt), Cofactors, & Inhibitors

- 5. All enzymes function optimally at PH 7.4

  True or **False**

Questions

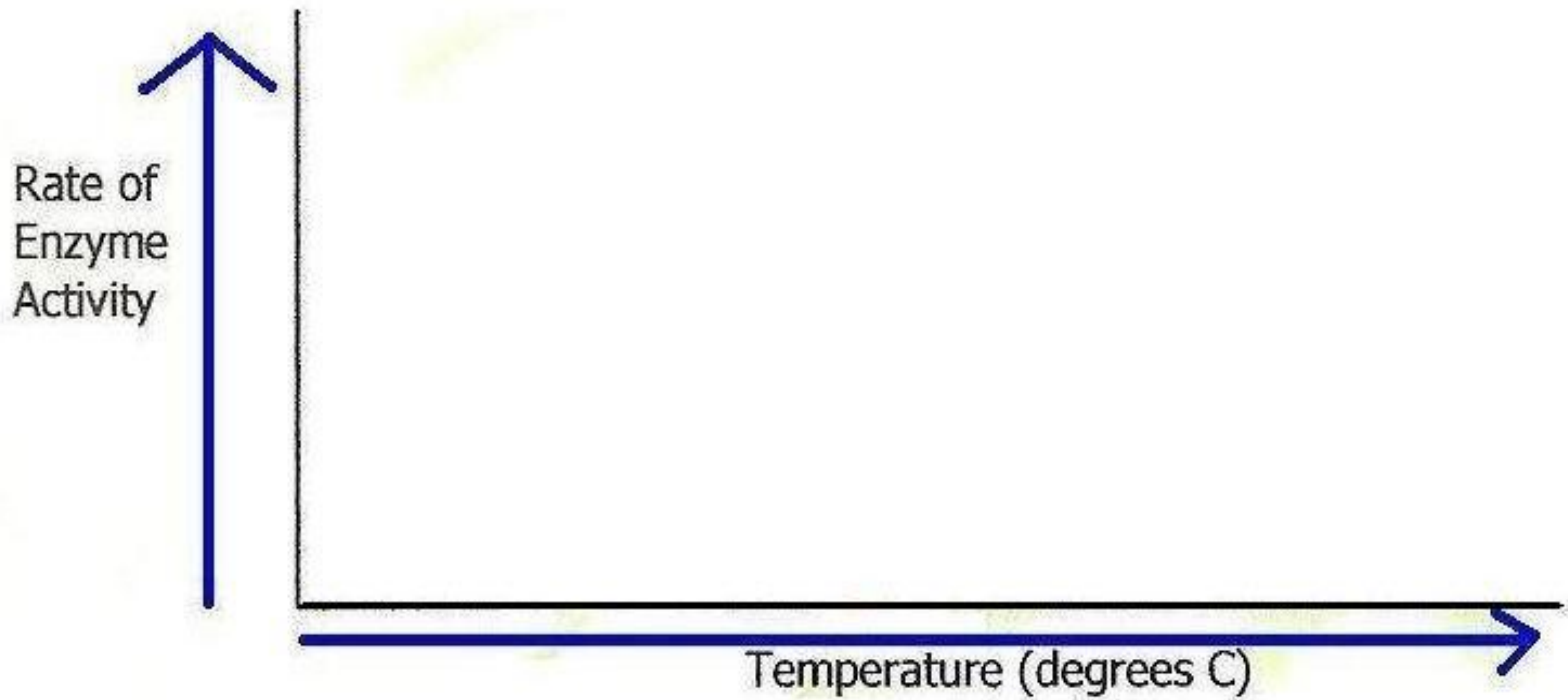
- 6. What is the importance of enzyme in living systems?
- 7. The optimum temperature of our enzyme was?
- 8. In general, as temperature increases, the rate of enzyme activity (increases/decreases) up to a certain point called the optimum temperature, at which activity is at a maximum. After this point, activity (increasing/decreases). At very high temperatures we see (high/low/no) activity because the enzyme is denatured.

Questions

- 6. What is the importance of enzyme in living systems? **Enzymes speed up chemical reactions by lowering Ea.**
- 7. The optimum temperature of our enzyme was **35 degrees Celsius.**
- 8. In general, as temperature increases, the rate of enzyme activity (**increases/**decreases) up to a certain point called the **optimum** temperature, at which activity is at a maximum. After this point, activity (increasing/**decreases**). At very high temperatures we see (high/low/**no**) activity because the enzyme is **denatured**.

Questions

- Draw a curve representing enzyme activity as it changes with temperature. Label the point of the optimum temperature.



Questions

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