7
Enzymes

Learning Objectives

In this chapter, you will learn:

- ➜ Enzyme Structure and Function
- ➜ Environmental Factors that Affect Enzyme Function
- ➜ Activation Energy in Chemical Reactions
- ➜ Energy and Metabolism/Coupled Reactions

Overview

Living cells are complex chemical factories that carry out the chemical reactions necessary to support life. These chemical reactions proceed at the rates required to support life because of the catalytic action of enzymes. This chapter will review the structure and function of enzymes and how environmental factors influence the rate of enzyme-catalyzed chemical reactions.

Enzyme Structure and Function

Many of the chemical reactions needed to support living systems happen too slowly to meet the changing needs of organisms. Catalysts speed up chemical reactions. **Enzymes** are biological catalysts. Most enzymes are made of proteins, which have a three-dimensional tertiary structure that is specific to their function. (**Ribozymes** are biological catalysts that are made of RNA.)

The **active site** of an enzyme interacts with the **substrate** (or reactant). The shape of the active site on the enzyme is specific to the shape of the substrate, as shown in Figure 7.1. The substrate must be able to fit into the active site to interact with the enzyme. If there are any charged R-groups on amino acids within the active site of the enzyme, there must be compatible charges on the substrate. For example, an active site that contained positively charged amino acids would repel any positively charged molecules, even if the molecule's shape could fit in the enzyme's active site.

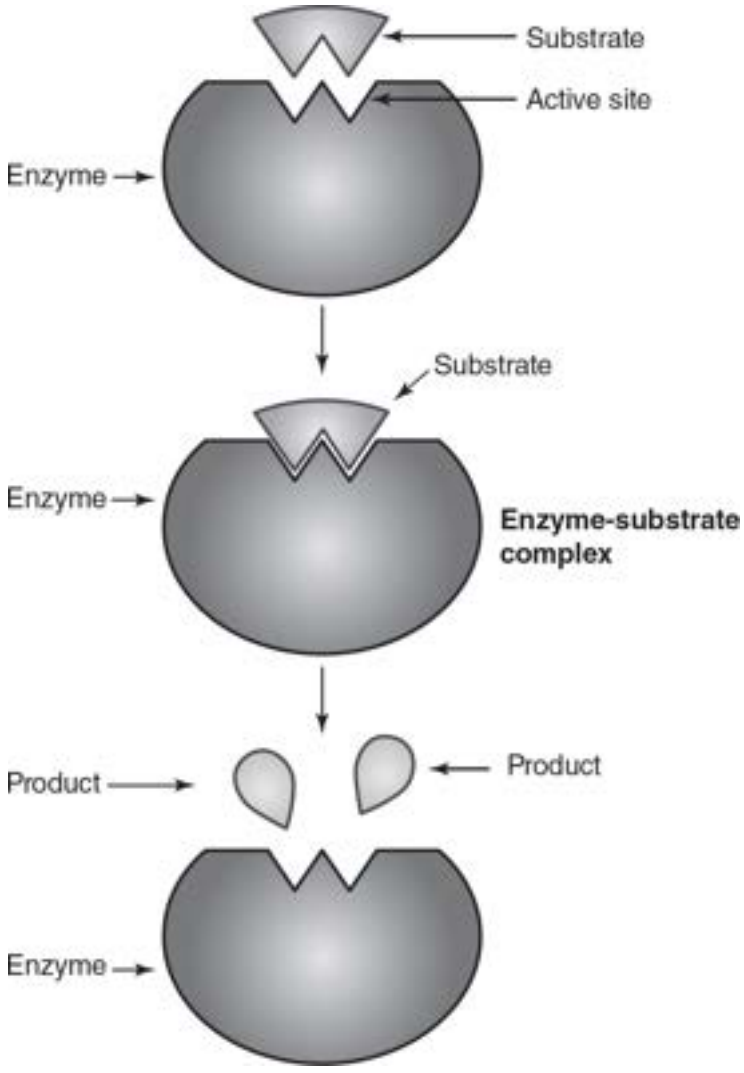

Figure 7.1 Enzyme Action

Environmental Factors that Affect Enzyme Function

Enzymes catalyze reactions most efficiently at optimum temperatures and pHs that are specific to the enzyme. If the temperature in the environment is too low, the rate of collisions between the enzyme and its substrate will be reduced, and the reaction will slow down. If the temperature is too high, bonds that hold the enzyme together may be disrupted, and the shape of the enzyme can be altered. Similarly, a pH that is too far from optimum can disrupt bonds in the enzyme and result in a change in its tertiary structure. Changes to the ionic environment of an enzyme can also disrupt bonds in the enzyme. A change to an enzyme's structure is called **denaturation**, and this can limit the enzyme's ability to catalyze chemical reactions. Sometimes, but not always, denaturation can be reversed when the environment returns to more optimum conditions.

Competitive inhibitors are similar in shape to substrates and compete with substrates for the active site of an enzyme, see Figure 7.2. This competition lowers the rate of enzyme-catalyzed reactions. The effect of competitive inhibitors can be diluted by adding higher concentrations of substrate, creating an environment where the substrate can outcompete the competitive inhibitor.

Noncompetitive (or **allosteric**) **inhibitors** do not bind to the active site but rather bind to a different site on the enzyme (called the **allosteric site**), see Figure 7.2. The binding of the noncompetitive inhibitor to the allosteric site changes the shape of the enzyme, affecting its function. Because the noncompetitive inhibitor does not bind to the active site of the enzyme, adding higher concentrations of substrate does not affect the action of a noncompetitive inhibitor. Noncompetitive inhibitors can function in feedback mechanisms, adjusting the rate of chemical reactions in the cell to suit changing environmental conditions.

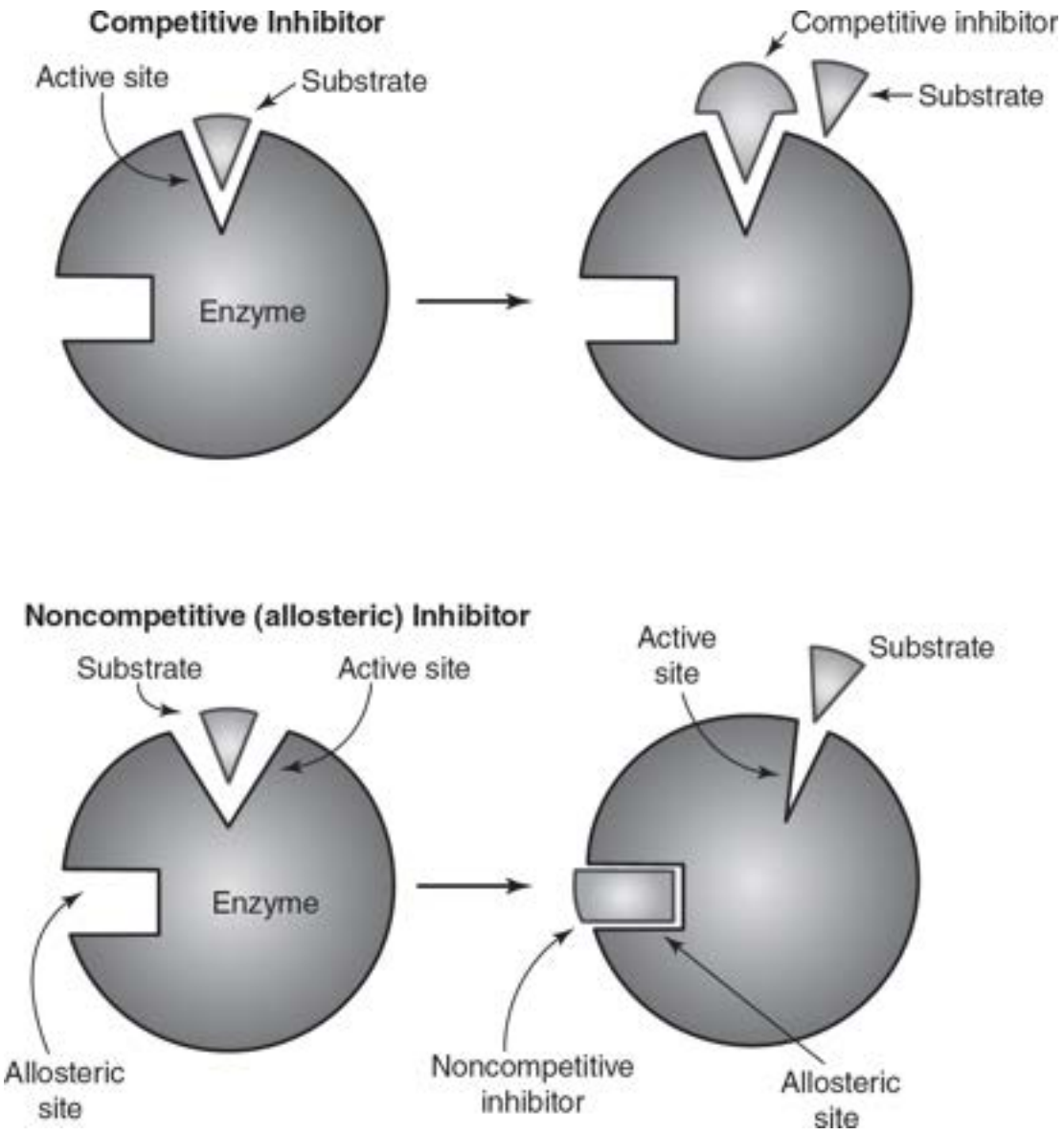

Figure 7.2 Competitive Inhibitors vs. Noncompetitive Inhibitors

Cofactors (inorganic molecules) and **coenzymes** (organic molecules) increase the efficiency of enzyme-catalyzed reactions, usually by binding to the active site or the substrate, which enhances the binding of the substrate to the active site.

Many of the vitamins and minerals in the foods you eat function as coenzymes and cofactors. For example, B vitamins help your body make the electron carrier NAD, which is important in cellular respiration, which will be discussed in Chapter 9.

Activation Energy in Chemical Reactions

All molecules have a given amount of free energy (G). The chemical reactions necessary for life involve changes in molecules. Chemical reactions can be endergonic or exergonic. **Endergonic** reactions have products with a higher free energy level than its reactants and are considered energetically unfavorable. **Exergonic** reactions have products with a lower free energy level than its reactants and are considered energetically favorable.

TIP

Think of activation energy as being analogous to a speed bump in a parking lot. The higher the speed bump is, the slower the car needs to proceed over it. The higher the activation energy, the slower the chemical reaction.

All chemical reactions require an input of energy to reach a transition state to get the reaction started. The **activation energy (E_A)** is the difference between the energy level of the reactants and the transition state of the reaction. (See Figure 7.3.) Higher activation energies result in slower chemical reactions; lower activation energies allow chemical reactions to proceed at a faster rate. The enzymes speed up chemical reactions by lowering the activation energy of the reaction.

Enzymes can lower the activation energy of a reaction in a number of ways:

1. Bringing substrates together in the proper orientation for a reaction to occur
2. Destabilizing chemical bonds in the substrate by bending the substrate
3. Forming temporary ionic or covalent bonds with the substrate

While enzymes can lower the activation energy of reactions, enzymes *cannot* change an endergonic reaction into an exergonic reaction. Enzymes

cannot change an energetically unfavorable reaction into an energetically favorable reaction.

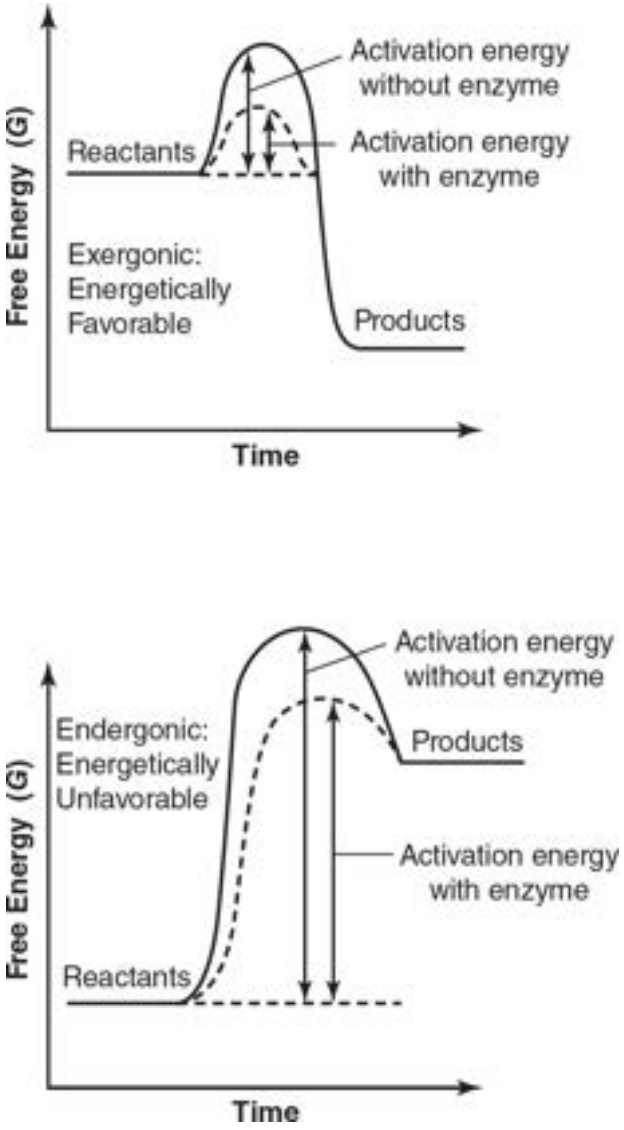

Figure 7.3 Reaction Profiles

Energy and Metabolism/Coupled Reactions

Life requires a constant input of energy to power cellular processes and maintain order in living systems. The energy input into the cell must be greater than the energy requirements of the cell in order to maintain life. Processes that release energy can be paired (or coupled) with processes that

require energy. These **coupled reactions**, as shown in Figure 7.4, occur in multiple steps to allow for the controlled transfer of energy between molecules, leading to more efficiency.

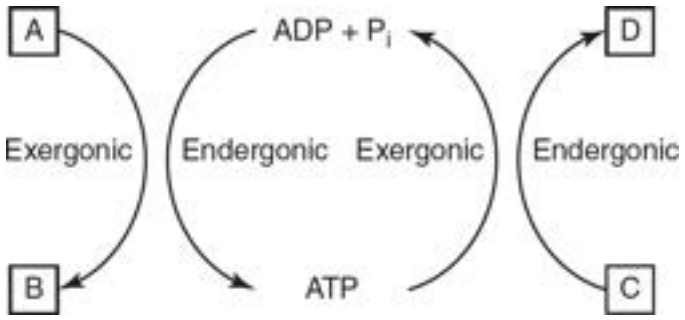

Figure 7.4 Coupled Reactions

Coupling an exergonic reaction with an endergonic reaction allows the energy released by the exergonic reaction to "drive" the endergonic reaction. For example, the breakdown of ATP into ADP and a phosphate group (P_i) is exergonic and releases approximately 30 kilojoules of energy per mole of ATP:

$$\text{ATP} \rightarrow \text{ADP} + \text{P}_\text{i} + 30\ \text{kJ}$$

The reaction that combines glucose and fructose to form sucrose requires approximately 27 kilojoules of energy per mole of sucrose formed:

$$\text{glucose} + \text{fructose} + 27\ \text{kJ} \rightarrow \text{sucrose}$$

Coupling these two reactions together shows that the exergonic breakdown of ATP into ADP releases more than enough energy to power the formation of sucrose from glucose and fructose:

$$\text{glucose} + \text{fructose} + \text{ATP} \rightarrow \text{sucrose} + \text{ADP} + \text{P}_\text{i} + 3\ \text{kJ}$$

Many of the endergonic chemical reactions that are required by living systems are powered by coupling them with exergonic reactions, such as the breakdown of ATP.

Practice Questions

Multiple-Choice

Questions 1–3

Catalase, an enzyme found in aerobic organisms, catalyzes the following reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

A filter paper disk is saturated with the enzyme catalase and then placed at the bottom of a beaker of H_2O_2. As the reaction proceeds, oxygen bubbles will cling to the paper and eventually the paper will float to the top of the liquid. By measuring the time it takes for the catalase-saturated disk to float, one can compare the relative rates of decomposition of H_2O_2 under different experimental conditions.

An experiment was performed using catalase extracted from potatoes, with varying concentrations of H_2O_2. Multiple trials were conducted, and the means and the standard errors of the mean are shown in the following table:

Concentration of H_2O_2	Mean Time for Disks to Float (seconds)	Standard Error of the Mean (seconds)
1%	92.6	6.2
3%	32.5	5.9
6%	15.1	4.5

1. Which of the following would be a suitable control for this experiment?

   (A) changing the temperature of the H_2O_2 in one of the beakers

   (B) changing the solution the paper disk is soaked in from catalase to water in one of the beakers

   (C) changing the source of the catalase from potato to liver

   (D) changing the pH of the H_2O_2 in one of the beakers

2. Predict what would happen if the catalase solution was boiled before performing the experiment, and justify your prediction.

   (A) The average time for the disks to float would increase because boiling denatured the enzymes.

   (B) The average time for the disks to float would increase because enzyme-catalyzed reactions are always slower at higher temperatures.

   (C) The average time for the disks to float would decrease because boiling increased the number of molecular collisions between the enzymes and substrates.

   (D) The average time for the disks to float would increase because enzyme-catalyzed reactions are always faster at higher temperatures.

3. A student graphs this data, showing 95% confidence intervals. Based on the 95% confidence intervals, which concentrations of enzyme are *least* likely to have statistically significant differences?

   (A) 1% and 3%

   (B) 1% and 6%

   (C) 3% and 6%

   (D) None of the enzyme concentrations used are likely to have statistically significant differences.

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4. BCR-ABL is an enzyme found in cancer cells in chronic myelogenous leukemia. ATP binds to the active site of BCR-ABL, which then stimulates cell division in cancer cells. Which of the following would most likely slow the rate of cell division in cancer cells with the BCR-ABL enzyme?

   (A) adding a cofactor of BCR-ABL

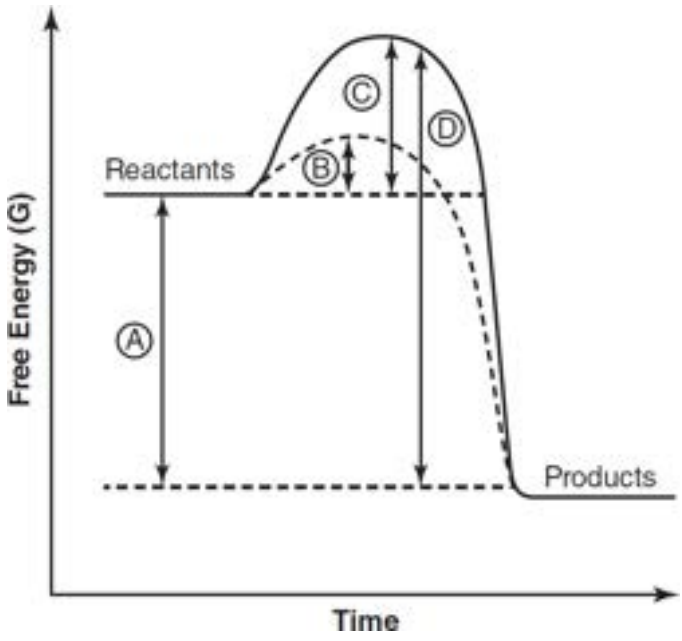
   (B) adding a coenzyme of BCR-ABL

   (C) adding a competitive inhibitor of BCR-ABL

(D) adding a transcription factor of BCR-ABL

Questions 5–7

Refer to the following figure.



5. Which of the following represents the activation energy of an enzyme-catalyzed reaction?

(A) A
(B) B
(C) C
(D) D

6. Which of the following represents the activation energy of an uncatalyzed reaction?

(A) A
(B) B
(C) C
(D) D

7. Which of the following represents the overall free energy change in the reaction?

(A) A
(B) B
(C) C
(D) D

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8. Which of the following correctly describes how enzymes increase the rate of a chemical reaction?

(A) Enzymes decrease the overall free energy change of the reaction.
(B) Enzymes decrease the activation energy of the reaction.
(C) Enzymes increase the free energy of the reactants of the reaction.
(D) Enzymes increase the free energy of the products of the reaction.

9. Which of the following correctly describes the differences between competitive inhibitors and noncompetitive inhibitors?

(A) Competitive inhibitors bind to the active site of the enzyme; noncompetitive inhibitors bind to the allosteric site of the enzyme.
(B) Competitive inhibitors bind to the substrate; noncompetitive inhibitors bind to the products.
(C) The effects of a noncompetitive inhibitor can be mitigated by adding large amounts of substrate.
(D) Competitive inhibitors increase the rate of enzyme-catalyzed reactions; noncompetitive inhibitors slow the rate of enzyme-catalyzed reactions.

10. Which of the following statements about enzymes is true?

(A) Enzymes can change endergonic reactions into exergonic reactions.
(B) Both proteins and RNA can have catalytic functions.
(C) Enzymes always function optimally at a pH of 7.

(D) Enzymes are consumed during chemical reactions.

Short Free-Response

11. Enzymes are important biological molecules.

    (a) **Describe** how an enzyme interacts with a substrate.

    (b) **Explain** the difference between competitive inhibitors and allosteric inhibitors of enzymes.

    (c) An enzyme has its maximum efficiency at an optimum temperature of 25° Celsius. **Predict** what effect a decrease in temperature to 5° Celsius would have on the enzyme's efficiency.

    (d) **Justify** your prediction from part (c).

12. The enzyme catalase breaks down hydrogen peroxide into water and oxygen, as shown in this equation:

$$2H_2O_{2\,(l)} \rightarrow 2H_2O_{\,(l)} + O_{2\,(g)}$$

An experiment was performed to measure the amount of oxygen bubbles produced at two different temperatures. The data are shown in the table.

Time (minutes)	Milliliters of Oxygen Produced at 37° Celsius	Milliliters of Oxygen Produced at 45° Celsius
0	0	0
5	2.5	0.5
10	5.2	0.8
15	7.7	1.0
20	10.3	1.1

(a) **Calculate** the rate of the enzyme-catalyzed reaction at both temperatures for the final 10 minutes of the experiment.

(b) **Predict** which temperature (37°C or 45°C) is closer to the optimum temperature for this enzyme.

(c) **Justify** your prediction from part (b) using the data provided.

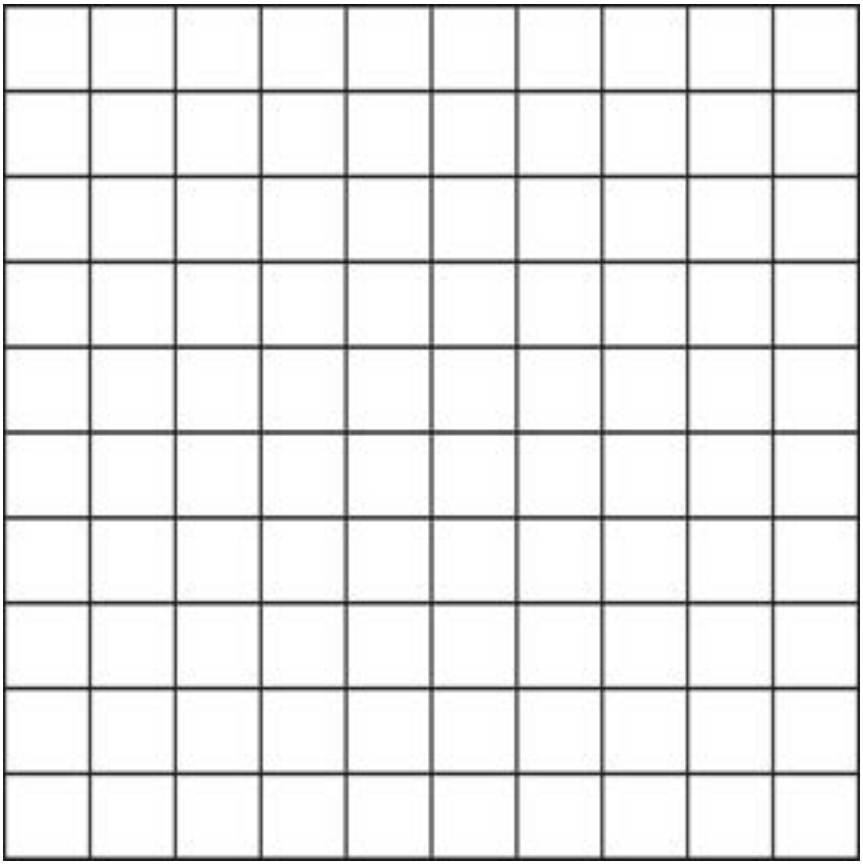
(d) In a follow-up experiment, the reaction was performed at a temperature of 50°C and no oxygen was produced. **Explain** why the enzyme would not function at 50°C.

Long Free-Response

13. Amylase is an enzyme that catalyzes the breakdown of amylose into its glucose subunits. The activity of amylase was measured at 37° Celsius and a pH of 7, both of which are optimum conditions for the activity of this enzyme. The production of glucose was measured both with and without the presence of compound X. Data are shown in the following table:

Time (min)	Cumulative Amount of Glucose Produced (millimoles) in the Absence of Compound X	Cumulative Amount of Glucose Produced (millimoles) in the Presence of Compound X
0	0	0
5	5.6	7.5
10	12.0	16.7
15	17.0	24.0
20	22.1	31.4

(a) On the axes provided, **construct** an appropriately labeled graph of this data.



(b) Based on the data and your graph from part (a), **identify** compound X as either: a cofactor, a competitive inhibitor, or a noncompetitive inhibitor of amylase. **Justify** your answer with evidence from the data.

(c) **Construct** an additional line on your graph from part (a) that represents your prediction as to the expected experimental results at a temperature of 10° Celsius in the absence of compound X.

(d) **Explain** your prediction from part (c), stating why you placed the additional line where you did.

Answer Explanations

Multiple-Choice

1. **(B)** Using a paper disk soaked in water would demonstrate the rate of reaction without the enzyme and would provide a basis for comparison. Choice (A) is incorrect because changing the temperature in just one of the beakers would introduce another variable into the experiment. Choice (C) is incorrect because this change would only lead to comparing the effectiveness of liver catalase versus that of potato catalase. Changing the pH in just one of the beakers would also introduce another variable into the experiment, so choice (D) is also incorrect.

2. **(A)** Boiling denatures most enzymes, making them ineffective, and thus the reaction time would increase. Choice (B) is incorrect because some enzymes may be more effective at higher temperatures (for example, enzymes found in bacteria that live in warm environments). While increasing the temperature does increase the number of molecular collisions, choice (C) is incorrect because boiling would denature the enzyme, making it an ineffective catalyst. Choice (D) is incorrect because if the rate of the enzyme-catalyzed reaction was faster at higher temperatures, the average time for disks to float would decrease, not increase.

3. **(C)** The upper limit of the 95% confidence interval for the 6% concentration of enzyme (15.1 + 2(4.5) = 24.1) is greater than the lower limit of the 95% confidence interval for the 3% concentration of enzyme (32.5 – 2(5.9) = 20.7), so their 95% confidence intervals overlap. When 95% confidence intervals overlap, it is not possible to say there is a statistically significant difference between the two groups. Thus, choice (C) is the correct answer. The 95% confidence intervals for the 1% and 3% enzyme concentrations do not overlap, so choice (A) is incorrect. Similarly, choice (B) is incorrect because the 95% confidence intervals for the 1% and 6% enzyme concentrations do not overlap. Choice (D) is incorrect because the 95% confidence intervals for the 1% and 3% and

the 1% and 6% enzyme concentrations do not overlap and likely have statistically significant differences between them.

4. **(C)** Competitive inhibitors bind to the active site of an enzyme. Therefore, adding a competitive inhibitor of BCR-ABL would block ATP from binding to its active site. Choices (A) and (B) are both incorrect because cofactors and coenzymes enhance enzyme function. Choice (D) is incorrect because a transcription factor would not affect ATP's ability to bind to the enzyme.

5. **(B)** The activation energy in the presence of an enzyme is the difference in free energy between the reactants and the transition state in the presence of the enzyme. Choice (A) is incorrect because it represents the overall free energy change of the reaction. Choice (C) is incorrect because it represents the activation energy of the reaction without the enzyme. Choice (D) represents the difference in free energy between the transition state of the reaction without the enzyme and the products of the reaction and is therefore incorrect.

6. **(C)** The activation energy in the absence of an enzyme is the difference in free energy between the reactants and the transition state of the higher activation energy. Choice (A) is incorrect because it represents the overall free energy change of the reaction. Choice (B) is incorrect because it represents the activation energy of an enzyme-catalyzed reaction. Choice (D) represents the difference in free energy between the transition state of the reaction without the enzyme and the products of the reaction and is therefore incorrect.

7. **(A)** The overall free energy change of the reaction is the difference between the free energy of the products less the free energy of the reactants. Choice (B) is incorrect because it represents the activation energy of an enzyme-catalyzed reaction. Choice (C) is incorrect because it represents the activation energy of the reaction without the enzyme. Choice (D) represents the difference in free energy between the transition state of the reaction without the enzyme and the products of the reaction and is therefore not the right answer.

8. **(B)** Enzymes increase the rate of reactions by reducing the activation energy of the reaction. Choice (A) is incorrect because enzymes never affect the overall free energy change of the reaction. Choices (C) and (D) are incorrect because enzymes cannot change the free energy of the reactants, nor can they change the free energy of the products of a reaction.

9. **(A)** This statement accurately describes where competitive and noncompetitive inhibitors bind on enzymes. Choice (B) is incorrect because neither competitive inhibitors nor noncompetitive inhibitors bind to substrates or products. Choice (C) is incorrect because adding substrate can mitigate the effects of a competitive inhibitor, not a noncompetitive inhibitor. Both types of inhibitors reduce, not increase, the rate of enzyme-catalyzed reactions, so choice (D) is also incorrect.

10. **(B)** Enzymes are made of protein, ribozymes are made of RNA, and both have a catalytic effect on chemical reactions. Choice (A) is incorrect because enzymes never affect whether a reaction is endergonic or exergonic. Not all enzymes have an optimum pH of 7, so choice (C) is incorrect. Choice (D) is incorrect because enzymes are never consumed in the reactions they catalyze.

Short Free-Response

11. (a) Enzymes interact with a specific substrate that has properties (such as shape and charge) that are compatible with those at the enzyme's active site.

    (b) Competitive inhibitors bind to enzymes at the active site, whereas allosteric inhibitors bind to enzymes at the allosteric site.

    (c) A decrease in temperature to 5° Celsius would decrease the enzyme's efficiency.

    (d) Decreases in temperature reduce the number of molecular collisions between the substrate and the enzyme, reducing the number of chemical reactions and the enzyme's efficiency. Decreases in

temperature may also alter the tertiary structure of the enzyme, altering its active site and reducing its catalytic ability.

12. (a) At 37°C, the rate of the enzyme-catalyzed reaction for the final 10 minutes of the experiment was $\frac{(10.3 - 5.2)\text{ milliliters}}{10\text{ minutes}} = 0.51\ \frac{\text{milliliters}}{\text{minute}}$. At 45°C, the rate of the enzyme-catalyzed reaction for the final 10 minutes of the experiment was $\frac{(1.1 - 0.8)\text{ milliliters}}{10\text{ minutes}} = 0.03\ \frac{\text{milliliters}}{\text{minute}}$.

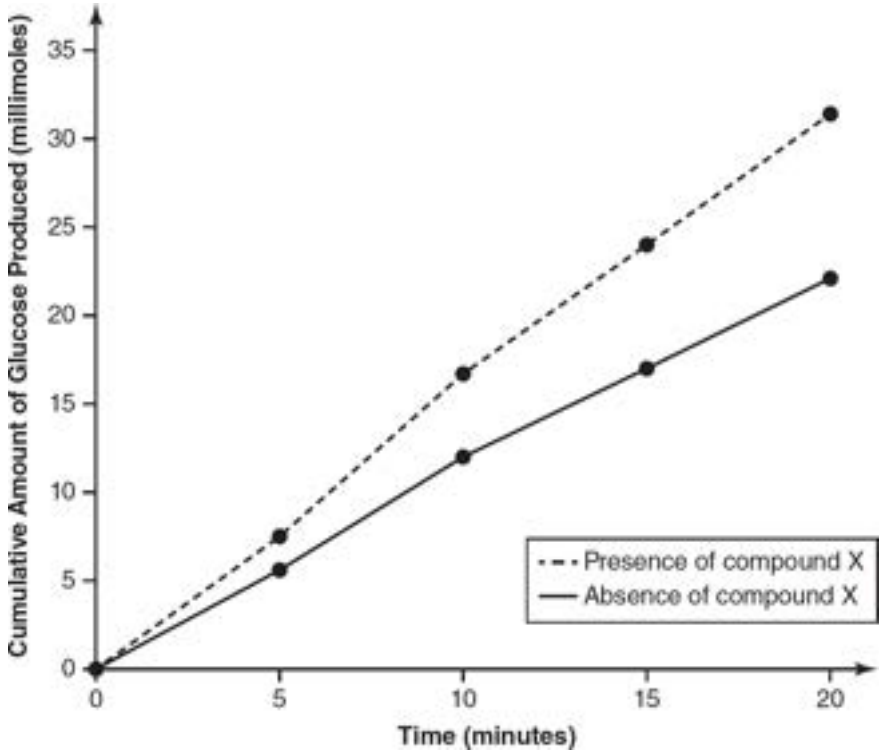
(b) The optimum temperature for this enzyme is closer to 37°C.

(c) The rate of the reaction is greater at 37°C than at 45°C, so 37°C is closer to the optimum temperature for this enzyme.

(d) At 50°C, the enzyme was probably denatured. High temperatures can denature enzymes, changing their shape so that they no longer function.

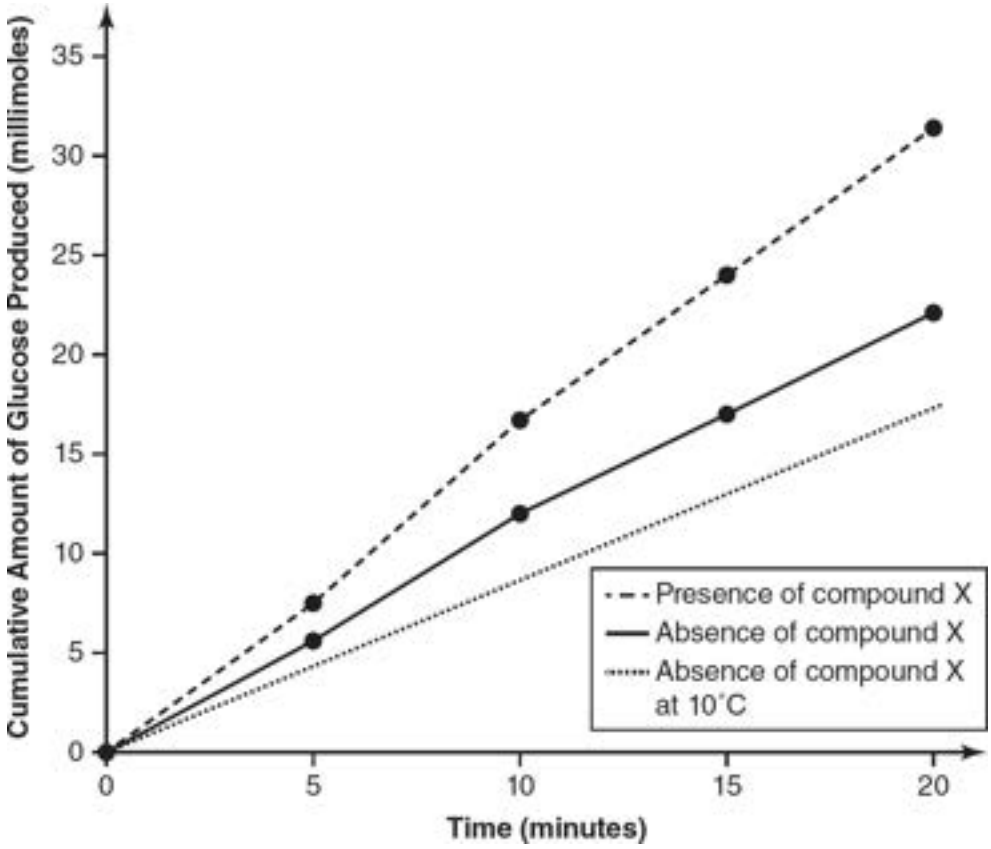
Long Free-Response

13. (a)



(b) Compound X is likely a cofactor. Cofactors increase enzyme efficiency. Because the amount of product produced in the presence of compound X is greater than the amount of product produced in the absence of compound X, compound X is most likely a cofactor of amylase.

(c)



(d) At reduced temperatures, there are fewer molecular collisions between the enzyme and the substrate as a result of the reduced kinetic energy at lower temperatures. Therefore, it is reasonable to predict that the reaction rate at 10°C would be slower than that of either 37°C measurement and thus that line is lower on the graph than the other two lines.