Key

Enzymes and Cellular Regulation

What are the factors that regulate the rate at which enzymes catalyze reactions?

Why?

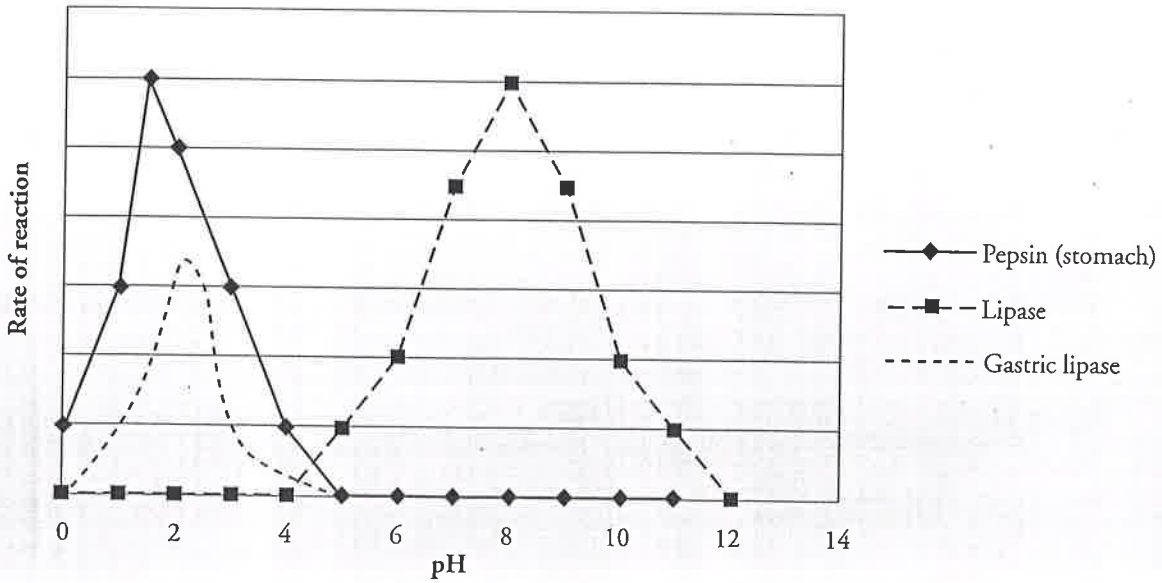
Digestive enzymes are protein-based biological catalysts that play important roles in our lives. They help remove stains from our shirts, turn milk into cheese, and are responsible for turning our dinner into useable fuel for our bodies. Enzymes however do not work well universally. Some are meant to work at high temperatures, others at low temperatures. They may work best in acidic conditions or neutral conditions. In this activity we will look at the optimal conditions for two different enzymes. The digestive enzyme lipase is made in the pancreas and breaks down lipids in the small intestine, while pepsin breaks down proteins in the stomach.

Model 1 – Two Digestive Enzymes

$$\text{Triglycerides} \xrightarrow{\text{lipase}} \text{glycerol + fatty acids}$$

$$\text{Large polypeptides} \xrightarrow{\text{pepsin}} \text{smaller polypeptides + amino acids}$$

Effect of pH on Enzyme Activity



1. Name the two enzymes illustrated in Model 1.

   Pepsin and lipase.

2. Consider the information provided in the *Why?* box and in Model 1 about these proteins.

   a. In which body organ is pepsin active?

   The stomach.

   b. In which body organ is pancreatic lipase active?

   The small intestine.

3. For each enzyme in Model 1, circle the pH that best represents the environment in which the enzyme is most active.

Pepsin	(1.5)	8	10.4
Lipase	1.5	(8)	10.4

4. Compare the rate of the pepsin-catalyzed reaction at pH 1.5 with the rate of the lipase-catalyzed reaction at pH 1.5.

   Pepsin is maximally active at pH 1.5, while lipase is inactive at pH 1.5

5. Compare the rate of the pepsin-catalyzed reaction at pH 8 with the rate of the lipase-catalyzed reaction at pH 8.

   Lipase is maximally active at pH 8, while pepsin is inactive at pH 8.

6. Using your knowledge of protein structure, explain in detail the effect of exposing an enzyme to a pH outside of its optimal range. Include the effect on both enzyme structure and function.

   A change in pH can alter the weak bonds and interactions that stabilize the secondary, tertiary, and quaternary structure of a protein. Since the function of an enzyme is based on the shape of the enzyme molecule (so that it will fit with the substrate), a change in shape due to denaturation would lower the rate of enzyme activity.

7. At what pH values is lipase likely to be denatured? Justify your answer.

   pH 0–4 and pH 12–14. At all of these pH values, lipase has no activity, so its shape is likely to be altered.

8. At what pH values is pepsin likely to be denatured? Justify your answer.

   pH 5–14. At these pH values, pepsin has no activity, so its shape is likely to be altered.

9. In addition to being produced in the pancreas, lipase is also produced in the stomach. Is the structure of pancreatic lipase the same as gastric (produced in the stomach) lipase? Justify your reasoning.

   Gastric lipase should have a lower optimal pH, one that is more similar to pepsin. Because gastric lipase has to function at a lower pH, it may have a different structure, so that it will retain it native folded structure, even at a lower pH.

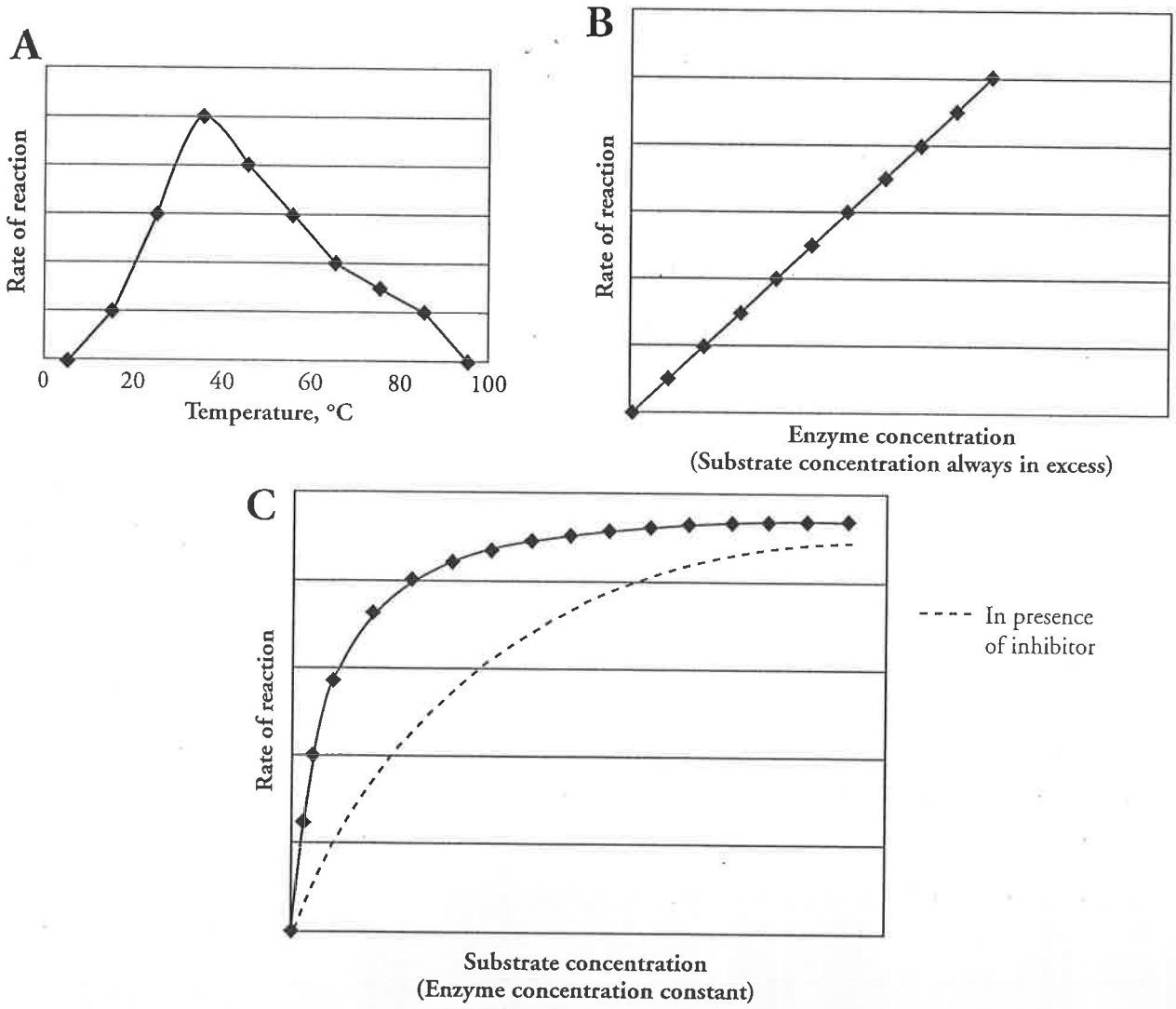
10. Add a line to the graph in Model 1 that shows a prediction for gastric lipase activity.

    See Model 1.

11. Antacids work by neutralizing acids, bringing the pH of the stomach to a range of 6–7. What is the effect of taking an antacid on a person's ability to digest proteins?

    Because the pH of the stomach will be higher, the pepsin will not work. The graph indicates that pepsin has no activity at pH 6–7. Therefore, no protein will be digested by pepsin when antacids have been used.

Model 2 – Amylase Rate of Reaction



12. Amylase is an enzyme that catalyzes the digestion of carbohydrates. The graphs in Model 2 provide data on several factors that affect the function of amylase in the body.

    a. The relationship of which two variables is illustrated in graph A of Model 2?

    Temperature and the rate of the enzyme-catalyzed reaction.

    b. The relationship of which two variables is illustrated in graph B or Model 2?

    Enzyme concentration and the rate of the enzyme-catalyzed reaction.

    c. The relationship of which two variables is illustrated in graph C or Model 2?

    Substrate concentration and the rate of the enzyme-catalyzed reaction.

13. Refer to Model 2.

    a. What is the optimum temperature for amylase?

    The optimal temperature is about 37 degrees Celsius.

    b. What is the biological significance of the temperature at which the amylase-catalyzed reaction is fastest?

    It is the temperature of the human body.

14. Predict what causes a decrease in enzyme activity at temperatures above 37 °C.

As the temperature increases past human body temperature (37 degrees Celsius), the enzyme's activity drops dramatically due to denaturation of the enzyme (protein) structure.

15. A young child runs a fever of 40 °C for 24 hours. Explain what effect this may have on his digestion.

Since the child's body temperature is above normal and above the optimum temperature for the digestive enzymes, the child's digestion may slow down since many of his digestive enzymes may be working more slowly or may even be denatured.

16. Consider the data in graph B of Model 2.

a. Describe the relationship between enzyme concentration and reaction rate.

As the enzyme concentration increases so does the rate of the reaction.

b. Propose an explanation for this relationship.

If there is more enzyme available to catalyze the reaction, then the reaction rate will increase.

17. Consider the data in graph C of Model 2.

a. What is the relationship between substrate concentration and the reaction rate?

As the concentration of substrate increases, the enzyme activity increases until the activity reaches a maximum value.

b. Propose an explanation for why a maximum reaction rate is reached in graph C.

If all of the enzyme molecules are bound to substrate and catalyzing the reaction, it does not matter how much substrate is present, the maximum rate has been achieved.

18. As a group, develop an analogy for the function of an enzyme that will explain the concentration graphs in Model 2 (graphs B and C).

Answers will vary. Possible answers include...

A matchmaker at a dance—More couples can be matched up when more matchmakers are at work, but at some point, the matchmakers are all busy, so the rate at which couples are made does not increase.

A lawnmower—A gas-powered mower will speed up the process of cutting the grass. More mowers will get the job done faster, but if the amount of grass is too large the mowers will not be able to cut it all before it grows tall again.

19. Would the reaction rate on graph B of Model 2 ever reach a maximum level? Justify your answer.

As more enzyme is added, the rate should increase as long as you have enough substrate for the enzyme to catalyze its reaction. However, if the substrate is also being added to the system, and that addition is slower than the enzyme rate of reaction, a maximum would be reached.

STOP

Extension Questions

20. Thermophilic bacteria, such as *Thermus aquaticus,* live in hot springs where the temperature is greater then 70 °C. Draw a graph similar to graph A in Model 2 representing the optimal temperature of *T. aquaticus.*

    Line drawn should show a peak at 70 degrees Celsius.

21. DNA polymerase from *T. aquaticus (Taq)* is used in PCR (polymerase chain reaction). PCR is a technique where millions of copies of DNA can be made from one original copy. In this method, the target DNA molecule is subjected to temperatures over 95 °C to make the double-stranded DNA separate. The temperature is then lowered slightly to allow primers to anneal before the *Taq* polymerase catalyzes the reactions to incorporate new nucleotides into the complementary strands. The cycle is then repeated over and over until there are millions of copies of the target DNA.

    a. Predict why this bacterial polymerase is used instead of a human polymerase.

    The Taq polymerase works optimally at higher temperatures, such as those found in thermal vents. The polymerase is less likely to be denatured at high temperatures.

    b. What would happen if you used a human polymerase in a series of PCR reactions?

    The human polymerase would be denatured at such high temperatures.

Read This!

The rate of an enzyme-catalyzed reaction can also be affected by the presence of other molecules that can bind to the enzyme, changing its shape. In some reactions a **coenzyme** is necessary. This molecule binds to the protein strands of the enzyme, changing its shape so that is ready to receive the substrate molecule. Without the coenzyme, the enzyme would not be able to attach to the substrate. Other molecules can reduce the rate of reaction for enzymes by binding to the protein and either blocking the spot where the substrate will bind or by making the enzyme's shape incompatible with the substrate. These molecules are called **inhibitors.**

22. Sketch a graph that shows the relationship between the rate of an enzyme reaction and the concentration of coenzyme necessary for the enzyme to function properly.

    The graph should be similar to graph B in Model 2—showing an increase in rate as the coenzyme concentration increases.

23. Add a line to graph C of Model 2 that shows the rate of an enzyme reaction in the presence of inhibitor molecules.

    See Model 2. The graph should be a similar shape, but all points should be lower than the present line. Note to teachers: In the presence of a competitive inhibitor, the maximum velocity does not change it just takes a lot more substrate to reach it.