

Enzyme Function

Objectives

Having completed this exercise, students should be able to:

1. explain the effects of temperature, pH, and substrate concentration on the performance of enzymes
2. apply the scientific method
3. communicate the results of your experiments in scientific form

Introduction

The liver is an important organ with many functions. As you learned earlier in the course, glycogen is a carbohydrate polymer made of glucose subunits; glycogen is used for energy storage in animals. One of the liver's functions is to store glycogen. Another role of the liver is detoxification. Hydrogen peroxide (H_2O_2) is produced in small amounts in the body but is very toxic. Since all your blood eventually runs through the liver, any H_2O_2 in the blood will be destroyed. This destruction of H_2O_2 is accomplished by an enzyme, catalase, in the liver peroxisomes.

Enzymes

Enzymes speed the rate of chemical reactions. A catalyst is a chemical involved in, but not consumed in, a chemical reaction. Enzymes are proteins that catalyze biochemical reactions by lowering the activation energy necessary to break the chemical bonds in reactants and form new chemical bonds in the products. Catalysts bring reactants closer together in the appropriate orientation and weaken bonds, increasing the reaction rate. Without enzymes, chemical reactions would occur too slowly to sustain life.

The functionality of an enzyme is determined by the shape of the enzyme. The area in which bonds of the reactant(s) are broken is known as the active site. The reactants of enzyme catalyzed reactions are called substrates. The active site of an enzyme recognizes, confines, and orients the substrate in a particular direction.

Enzymes are substrate specific, meaning that they catalyze only specific reactions. For example, proteases (enzymes that break peptide bonds in proteins) will not work on starch (which is broken down by the enzyme amylase). Notice that both of these

enzymes end in the suffix -ase. This suffix indicates that a molecule is an enzyme. Environmental factors may affect the ability of enzymes to function. You will design a set of experiments to examine the effects of temperature, pH, and substrate concentration on the ability of enzymes to catalyze chemical reactions. In particular, you will be examining the effects of these environmental factors on the ability of catalase to convert H_2O_2 into H_2O and O_2 .

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The Scientific Method

As scientists, biologists apply the scientific method. Science is not simply a list of facts, but is an approach to understanding the world around us. It is use of the scientific method that differentiates science from other fields of study that attempt to improve our understanding of the world.

The scientific method is a systematic approach to problem solving. Although some argue that there is not one single scientific method, but a variety of methods; each of these approaches, whether explicit or not, tend to incorporate a few fundamental steps: observing, questioning, hypothesizing, predicting, testing, and interpreting results of the test. Sometimes the distinction between these steps is not always clear. This is particularly the case with hypotheses and predictions. But for our purposes, we will differentiate each of these steps in our applications of the scientific method.

Observation

An observation is something that the scientist notices. This may be a direct observation using one or more of his or her senses or it may be an observation made by someone else that has been communicated to the scientist. An everyday example might be "when I flip the light switch, the light does not come on."

Question

The observation leads to a question. In the case of our example, an obvious question is "why won't the light come on?"

Hypothesis

An hypothesis is often described as an educated guess. It is a possible answer to the

question that has been asked. It is inevitable that there is more than one possible answer. If multiple answers are proposed, these are referred to as alternative hypotheses. In our example, an hypothesis might be that "the light bulb is dead." Alternative hypotheses might include "the power is out," "the circuit is broken," or "the fuse has been tripped." Hypotheses must be testable and falsifiable. If the hypothesis is wrong, we must have an opportunity to demonstrate that it is false.

Prediction

Predictions bridge the hypothesis and the test. The prediction tends to be more specific than the hypothesis and is often worded as an "if, then" statement. "If the light bulb is dead, then when I replace it with a new bulb, the light should come on."

Test

The test is what is actually done to evaluate the prediction. The test involves the collection of data using one or more of our five senses. Tests may involve manipulative experiments or observational studies. In our example, we are manipulating the conditions by changing the light bulb. When we flip the switch, we observe with our eyes whether the light comes on. Observational studies do not involve direct manipulation of

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the testing conditions by the scientist. Observational studies are often used when manipulative experiments cannot be conducted or if the experiment would be unethical to conduct. For example, to test the prediction that lead affects the mental development of children, it would be unethical to expose a group of randomly selected kids to lead. Rather, the scientist is likely to take blood samples, measure the lead content, and compare lead concentrations with test scores evaluating mental aptitude.

Interpret Results

After observing the results of the test, the scientist must evaluate what the results mean. The results will be used to either reject the hypothesis, or they will fail to reject the hypothesis. If the light does not come on, we reject the hypothesis. This does not mean that the experiment has failed. In fact, it means that the scientific method has worked just as it should. Often, this leads to new hypotheses and/or testing of alternative

hypotheses.

Procedures

Observe the Effects of Catalase

1. Obtain two small pieces of liver (10 to 20 mm long).
2. Place the liver into each of two test tubes marked 'A' and 'B'.
3. Squeeze enough H₂O₂ from the

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What was the purpose of the water in tube 'B'?

4. Since the enzymes are not used up, transfer the liquid to a clean tube. Add more H₂O₂ to the tube containing the liver.

What happened in the tube?

5. To the liquid from the first tube, add several drops of fresh H₂O₂.

Was there a reaction? Explain.

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Effects of pH, Temperature, and Substrate Concentration

Observations

From the introduction and your reading, you have some background knowledge on enzyme structure and function. You also just observed the effects of catalase on the reaction in which hydrogen peroxide breaks down into water and oxygen.

Questions

From the objectives of this lab, our questions are as follows:

1. How does temperature affect the ability of enzymes to catalyze chemical reactions?
2. How does pH affect the ability of enzymes to catalyze chemical reactions?
3. What is the effect of substrate concentration on the rate of enzyme catalyzed reactions?

Hypotheses

Based on the questions above, come up with some possible hypotheses. These should be general, not specific, statements that are possible answers to your questions.

Predictions

Based on your hypotheses, make some predictions about what you expect to see in your test results. This will require a little knowledge about the experimental setting that you have available. In addition to the liver and hydrogen peroxide, you will have ice and a hot plate available to warm (not boil) water. You will also have several pH buffers to create acidic, neutral, and basic environments. Write your predictions as if/then statements in the space provided below.

Results: Test Your Hypotheses

Based on your predictions, design a set of experiments to test your hypotheses.

Interpret your results to draw conclusions

Do your results match your predictions?

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Do the results reject or fail to reject your hypothesis and why?

What might explain your results? If your results are different from your predictions, hypothesize why they may differ. If the results matched your predictions, hypothesize some mechanisms behind what you have observed.

Communicating Your Findings

Scientists generally communicate their research findings in written reports. Save the things that you have done above. You will be use them to write a lab report a little later in the course.

Sections of a Lab Report

Each report should always start with a title and a byline. The title describes the focus of the research. The byline lists the authors of the paper.

Abstract

The abstract is an overall summary of the paper. It is generally short (<250 words) and should include a sentence or two introducing the problem, describing the methods, summarizing the results, and discussing the significance of the results.

Introduction

The introduction provides the reader with background information about the problem

and provides the rationale for conducting the research. The introduction should incorporate and cite outside sources. You should avoid using websites and encyclopedias for this background information. The introduction should start with more

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broad and general statements that frame the research and become more specific, explaining your hypotheses and predictions near the end.

Methods

The methods section describes how the study was designed to test your hypotheses. This section should provide enough detail for someone to repeat your study. This section explains what you did. It should not be a bullet list of steps and materials used; nor should it read like a recipe that the reader is to follow.

Results

This section provides a written description of the data. This section should also include numbered graphs or tables with descriptive captions. The objective is to present the data, not interpret the data.

Discussion

In this section you interpret and critically evaluate your results. Generally, this section begins by reviewing your hypotheses and predictions, and whether your data support your hypotheses. In describing conclusions that can be drawn from your research, it is important to include outside studies that help clarify your results. What is most important about the research? What is the take-home message?

References

This section should list all scientific articles or books that were used in the introduction and discussion. All references listed must be cited within the text at the point where they are used. Format (MLA, APA, etc.) is unimportant as long as it is detailed enough for me to find the original source of information. Wikipedia, About.com, etc. should not be used as references.

Example lab reports will be provided along with more detailed descriptions of each section, if needed.

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Enzyme Function

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The translation was initiated by Scott Rollins on 2011-07-22