Enzyme Activity

**In addition to close-toed shoes, you are required to wear long pants or skirt (ankle length) to participate in this lab. You will not be allowed in the lab without these minimum requirements.**

Objectives

After completing this exercise you should be able to:

1. Define catalyst, enzyme, active site, substrates, enzyme-substrate complex, products, synthesis, degradation, oxidation/reduction, isomerization, and enzyme specificity.

2. Explain how enzymes work.

3. Describe the experiment using sucrase and interpret the resulting data.

4. Explain the use of Benedict’s solution in demonstrating sucrase activity.

5. Describe how temperature and pH affect sucrase activity.

Introduction

**Enzymes** are usually protein molecules that act as biological catalysts. A catalyst greatly increases the speed of a chemical reaction by lowering the activation energy necessary to get the reaction started without itself being altered or consumed.

On the surface of the enzyme is an **active site** that temporarily binds the reactants or **substrates** forming an **enzyme-substrate complex**. The catalytic action of the enzyme then converts the substrate to a **product** or **products**. This conversion can take the form of a synthesis (building more complex molecules), a **decomposition** (splitting of the substrate), an **oxidation/reduction** (addition or removal of electrons), or an **isomerization** (rearrangement of atoms within a molecule).

When the product or products are released, the enzyme emerges unchanged and available to convert more substrate into more products. Since enzymes can be used again and again, they are effective even at low concentrations.

Each enzyme is highly **specific**; that is, it catalyzes only a single chemical reaction or small group of related reactions. An enzyme can distinguish its substrate from even closely related isomers. For example, the enzyme maltase will catalyze the breakdown of the disaccharide maltose into its two glucose subunits. However, the disaccharides sucrose and lactose are unaffected by maltase.
The activity of an enzyme is affected by many factors including temperature and pH. Each enzyme has an optimum temperature at which its reaction rate is fastest. For humans, the optimum temperature is 37°C, but for bacteria living in hot springs, the optimal temperature would be about 85°C.

**Temperature and pH Effects on Sucrase Activity**

The enzymatic hydrolysis of sucrose by the enzyme sucrase will result in the formation of its two monosaccharide components, glucose and fructose, as follows:

\[
\text{sucrase} \quad \text{Sucrose} + \text{water} \rightarrow \text{glucose} + \text{fructose}
\]

Or

\[
\text{sucrase} \quad \text{C}_{12}\text{H}_{22}\text{O}_{11} + \text{H}_{2}\text{O} \rightarrow \text{C}_{6}\text{H}_{12}\text{O}_{6} + \text{C}_{6}\text{H}_{12}\text{O}_{6}
\]

In this exercise you will conduct an experiment to test the role of sucrase in the digestion of sucrose. You will also examine the effects of temperature and pH on sucrase activity. Sucrase is present in a wide range of organisms including humans and even yeast. The sucrase stock solution to be used in this exercise was prepared by placing dry yeast cells in warm water for 10 minutes which allowed them to become metabolically active. The yeast suspension was then homogenized in a blender for three minutes which ruptured the cells and released sucrase into solution. The homogenate was filtered through cheesecloth and the resulting filtrate became the sucrase stock solution.

**Experimental Procedure**

To begin the exercise, obtain four clean test tubes and number them 1-4 with a marking pencil. Then, pipet the following solutions into their respective test tubes:

- **Tube 1:** 3 ml water
- **Tube 2:** 3 ml enzyme stock solution
- **Tube 3:** 3 ml enzyme stock solution
- **Tube 4:** 3 ml enzyme stock solution

Tubes 1 and 2 will received no further treatment, but tubes 3 and 4 will be treated with different reagents or placed at different temperatures in order to assess the effects of pH
or temperature on sucrase activity. Expose tubes 3 and 4 to only one of the following conditions for each tube: a boiling water bath for 10 minutes, an ice bath for 10 minutes, 10 drops of concentrated HCl, 10 drops of lemon juice, 10 drops of concentrated ammonium hydroxide (NH₄OH). If you choose to place a tube in the boiling water bath, be sure to use a test tube holder.

Formulate a hypothesis for your chosen experimental procedure. Write it here:

_____________________________________________________________________
_____________________________________________________________________
_____________________________________________________________________

After you have treated tubes 3 and 4, allow everything to return to room temperature and pipet 2 ml of a 5% sucrose solution into each of the four test tubes. Swirl each tube to mix its contents, and allow the tubes to stand at room temperature for 10 minutes.

Next you will test the contents of each tube for glucose and fructose using Benedict’s reagent. Benedict’s reagent is a blue solution of sodium bicarbonate, sodium citrate, and copper sulfate. When this solution is mixed and heated with a monosaccharide like glucose or fructose, a precipitate forms whose color will vary from green to red depending upon the quantity of monosaccharide present.

Using a “100-1000 μl” pipetman, transfer 5 ml (5 x 1,000 μl) of Benedict’s reagent into each tube, mix, and heat in a boiling water bath for two minutes. Using a test tube holder, carefully remove the four test tubes from the water bath. Estimate the relative amount of glucose and fructose present in the tubes by using the following scale:

<table>
<thead>
<tr>
<th>Blue</th>
<th>Green</th>
<th>Yellow</th>
<th>Orange</th>
<th>Red</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td>++++</td>
</tr>
</tbody>
</table>

Record the results in the following table.

<table>
<thead>
<tr>
<th>Tube</th>
<th>Color of precipitate</th>
<th>Amount of glucose &amp; fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Answer the following questions:

Which tubes showed sucrase activity? _____________________________

Why was tube 1 included in this experiment? _________________________

Why was tube 2 included in this experiment? _________________________

Describe the conditions (pH and temperature) under which sucrase was operating in tube 2.___________________________________________

_______________________________________________________________________

How do the results from tubes 3 and 4 compare with those from tubes 1 and 2?  
_______________________________________________________________________

_______________________________________________________________________

Was your hypothesis supported? _________________________

How can these results be explained in light of the fact that sucrase is a protein?  
_______________________________________________________________________

_______________________________________________________________________