Unit 6: Review
Enzymes Lab

Chemical Equation

I. Preparation of standard tubes

Potato extract contains the enzyme, catechol oxidase. This is prepared by peeling and dicing a small potato then blending it with water.

The 1% catechol is prepared in lab from a stock bottle.

Tubes were prepared to determine product concentration for specificity, temperature, pH, and cofactor necessity sections.

Tubes A-C were prepared and placed in a 40°C water bath for 20 minutes. Tube A and B are the blank tubes and Tube C is the experimental tube.

Tube A: potato extract + water
Tube B: 1% catechol + water
Tube C: potato extract + 1% catechol

To measure the amount of product contained in each test tube, you recorded either:
1. color intensity of the tube based on a set of standards
2. absorbance using a spectrophotometer

II. Enzyme specificity

Enzymes exhibit specificity. They are globular in shape and have one or more active sites, each of which is structured so that it bonds with a specific substrate.

Examine the structure of catechol in your lab manual. It resembles the hydroquinone, but they each have a hexagon ring shape with an OH group at the top. They differ in the location of the second OH group.

We will test both to see which substrate reacts better with catechol oxidase to produce benzoquinone.

1% hydroquinone

Enzyme specificity continued

Tubes were prepared and placed in 40°C water bath for 20 minutes.

Tube D: potato extract + 1% catechol
Tube E: potato extract + 1% hydroquinone

Observe the color intensity of the tube or the absorbance of each tube at 20 minutes.

Based on the color intensity of the tube or the absorbance reading, which substrate reacts better with catechol oxidase to produce benzoquinone? Does catechol oxidase express specificity?
Reactions catalyzed by enzymes have a temperature range in which the reaction will occur at an optimum level. Within this range, the reaction rate increases 10% per every 1°C increase in temperature. Temperatures well above or below this range can change the structure of the enzyme. When the structure of the enzyme changes, it is denatured and will no longer act as a catalyst.

The following temperatures were tested:
5°C, 25°C, 40°C, 60°C, 80°C, and 100°C.

III. Temperature and enzyme activity

Examine the following test tubes. Observe the color intensity of each tube at 20 minutes or record its absorbance. Plot your results on the graph and connect the dots.

At what temperature does catechol oxidase work best?

Temp. at 20 min:
5°C 25°C 40°C 60°C 80°C 100°C

IV. PH and enzyme activity

pH is a measure of the hydrogen ion concentration in a solution. Generally, most enzymes function best at a pH near neutral (pH 6-8). Enzymes are adapted to the environment in which they are active. For example, enzymes that are active during chemical digestion in the stomach function at a lower pH than those active in the small intestine.

The following pH buffers will be used:

Examine the following test tubes. Observe the color intensity of each tube at 20 minutes or record its absorbance. Plot your results on the graph and connect the dots.

At what pH does catechol oxidase work best?
Cofactors are inorganic compounds, usually metallic ions, that are a part of the active site of enzymes. Cofactors make the formation of an enzyme-substrate complex possible. Copper is a cofactor for catechol oxidase. This exercise is to determine whether copper is essential for catechol oxidase to produce benzoquinone. EDTA (ethylenediaminetetraacetic acid) binds copper and makes it unavailable for use as a cofactor.

### V. Cofactor necessity

<table>
<thead>
<tr>
<th>Tube F</th>
<th>Tube G</th>
</tr>
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<tbody>
<tr>
<td>contains catechol + potato extract</td>
<td>contains catechol + potato extract + EDTA</td>
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</table>

Observe the color intensity of each tube at 20 minutes or record the absorbance. Is the cofactor (Cu⁺⁺) necessary? In other words, does catechol oxidase need its cofactor to function?

End of Lab Review ☺

Cofactor necessity continued

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Continue