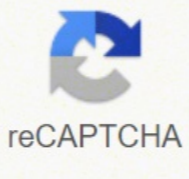




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needed will be reduced as well as the expensive equipment required (Schnell, 2000). Also enzymes are meant for domestic use. For instance, in "biological" detergents.

Materials and Methods

The experiment was carried out in pairs of two and the measurement got was compared with the average of the class measurement. The entire substrate and pH range was used as shown below. It was expected that the reaction to be set up then monitor the reaction with spectrophotometer for 5 minutes upto the point when the absorbance reading atleast 0. The readings were recorded after every 30 seconds. The was following conditions were investigated

Substrate concentrations: 4mM, 2mM, 1mM, 0.5 mM, 0.25mM, 0.125mM, 0.062mM, 0.031mM and blank  
pH: 3.5, 4.5, 5.5, 6.5, 7.5, and 8.5

As mentioned above, at the end of the experiment, the pair measurements was compared with the class average measurements and regression line was plotted to ascertain this comparison.

Each reaction had the following component

Ingredient	Volume
Assay buffer	600µL
β-gal 10x	Volume to attain 0.25U/ml concentration
ONPG	Volume required to attain a reaction concentration
H <sub>2</sub> O	To complete the volume to 1200µL
Reaction volume	<b>1200 µL</b>

Table 1: showing the reaction component.



### Kinetic Mechanisms of Tyrosinase Inhibitors in the Common White Mushroom (*Agaricus bisporus*) with Tropolone.

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November 5<sup>th</sup>, 2014

#### Introduction

A kinetic study of the inhibition of the enzyme tyrosinase, as found in the different parts of the common white mushroom *Agaricus bisporus*, by tropolone was conducted. The enzyme kinetic parameters  $V_{max}$ ,  $K_M$ , and  $V_{max}/K_M$  were determined by varying catechol concentrations, and thus, substrate concentrations with the enzyme-catalyzed reaction. The effect of changing either enzyme or substrate concentrations on initial reaction rate was analyzed. Modern scientists estimate kinetics parameters from experimental kinetics data using non-linear regression. However, the kinetic parameters that described the inhibition by tropolone were evaluated by using the Eadie-Hofstee equation. Linear transformation was used to study the inhibition mechanism of the tropolone in tyrosinase from the specific mushroom tissue and compare it to a published  $K_i$  value for the same inhibitor. The  $K_M$  of tyrosinase from the specific mushroom tissue was compared to its literature value.

Tyrosinase is a copper-containing enzyme present in microorganisms, plants and animals.<sup>[1]</sup> In fungi, tyrosinase catalyzes the rate-limiting step in the formation of the pigment melanin from tyrosine.<sup>[2]</sup> Tyrosinase catalyzes oxidations of tyrosinase to L-3,4-dihydroxyphenylalanine (L-DOPA), the substrate, to o-dopaquinone then to dopachrome, the primary pigment found in human red hair.<sup>[2]</sup>



Tyrosinase is also responsible for the browning of potatoes and many other foods. This is because tyrosinases catalyze the oxidation of monophenols and o-diphenols to o-quinones, the precursor compounds of the brown-colored pigment melanin.<sup>[3]</sup> Melanins are heterogeneous polymers of polyphenolic character and little defined structure with color typically ranging from yellow to dark brown.<sup>[4]</sup> Melanins originate the enzymatic browning in fruits and vegetables as well as the pigmentation of animals.<sup>[5]</sup> Tyrosinase has been ascribed other functions apart from melanin production, including the detoxification of host plant defensive phenols for symbiotic bacteria, the sclerotisation of insect cuticles, and the syntheses of amino acid based antibiotics.<sup>[2]</sup>

A complication of working with enzymes is that they can exist in different forms: isomers. It is unknown how many isomers of tyrosinase exist in the common white button mushroom, and multiple isomers of tyrosinase exists in *Agaricus bisporus*. However, the protein concentration of the mushroom extract for the experiment was measured and the tyrosinase isomers were identified using gel electrophoresis. In the cell, tyrosinase is activated by hydrolysis catalyzed by an unknown protease. Thus, tyrosinase is activated by the detergent sodium dodecyl sulfate (SDS) for this experiment.

Name: \_\_\_\_\_  
Date: \_\_\_\_\_

Experiment 10  
Enzyme Kinetics: Michaelis-Menten Kinetics and Lineweaver-Burk Plots

Read all the instructions before starting the experiment.

- Prepare the following solutions:
- Prepare the substrate solution (0.1 M catechol, 0.1 M phosphate buffer, pH 7.0, 0.1 M NaCl, 0.1 M MgCl<sub>2</sub>).
- Prepare the enzyme solution (0.1 M phosphate buffer, pH 7.0, 0.1 M NaCl, 0.1 M MgCl<sub>2</sub>, 0.1 M K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>).
- Prepare the inhibitor solution (0.1 M phosphate buffer, pH 7.0, 0.1 M NaCl, 0.1 M MgCl<sub>2</sub>, 0.1 M K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, 0.1 M tropolone).

Procedure

- Prepare the reaction mixture (0.1 M phosphate buffer, pH 7.0, 0.1 M NaCl, 0.1 M MgCl<sub>2</sub>, 0.1 M K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, 0.1 M tropolone, 0.1 M catechol, 0.1 M enzyme solution).
- Measure the initial reaction rate (absorbance at 420 nm) for each substrate concentration.
- Calculate the initial reaction rate (V<sub>0</sub>) for each substrate concentration.
- Plot the initial reaction rate (V<sub>0</sub>) versus substrate concentration (S<sub>0</sub>).
- Calculate the Michaelis-Menten constants (K<sub>M</sub> and V<sub>max</sub>) from the plot.
- Repeat the experiment for different inhibitor concentrations.
- Plot the initial reaction rate (V<sub>0</sub>) versus substrate concentration (S<sub>0</sub>) for different inhibitor concentrations.
- Calculate the inhibition constants (K<sub>i</sub>) from the plots.

Check Questions to test your experimental skills.

1. Calculate the initial reaction rate (V<sub>0</sub>) for each substrate concentration.

2. Calculate the Michaelis-Menten constants (K<sub>M</sub> and V<sub>max</sub>) from the plot.

3. Calculate the inhibition constants (K<sub>i</sub>) from the plots.



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