Amylase Activity Experiment

Adapted from "Forensics Enzymology: Can Enzymes Be A Detective's Best Friend?" by EDVOTEK® and "Kinetic Analysis of Amylase Using Quantitative Benedict's and Iodine Starch Reagents" by Beverly Cochran, Deborah Lunday, and Frank Miskevich.

Enzymes as Biological Catalysts

A biological catalyst is used in trace amounts to accelerate the rate of a biochemical reaction without being consumed or transformed during the reaction. Catalysts do not alter the equilibrium constant of reactions. Only the rate of approach to equilibrium is changed. Reactions in cells are catalyzed by biological catalysts known as enzymes which can accelerate reactions by as much as 10^{14} to 10^{20} times. Enzymes function best under physiological conditions at neutral pH, and temperatures of 37° C. Enzymes are generally very specific for the reactions they catalyze.

Measuring Enzyme Activity

The reactant molecule in an enzyme-catalyzed reaction is called the substrate. The substrate (S) is transformed to product (P). In this experiment, Amylase is an enzyme (E), which catalyzes the hydrolysis of the polysaccharide starch (S) to the disaccharide maltose (P). Salivary amylase is produced by the salivary glands. If amylase is added to a solution of starch, the starch will be digested to form maltose.

Substrate (S)	Enzyme (E)	Products (P)
Starch	————>	Maltose
	Amylase	

The appearance of product (P) or the disappearance of substrate (S) can be measured as a function of time during a reaction. One can measure the amount of product formed or the decrease in substrate at regular intervals. This quantity can be plotted as a graph.

Humans produce 1 to 1.5 liters of saliva per day. Saliva has a slightly alkaline pH and is composed of water, mucus, proteins, salts and enzymes. Saliva is used to lubricate food, to assist in swallowing and initiate food digestion by the enzyme amylase, which initiates digestion of starch. Amylases are ubiquitous and are found both in plants and animals. The enzyme is abundant in human saliva in easily detectable quantities. Amylase acts within the starch chains to produce small disaccharides known as maltose. The level of saliva amylase increases due to sleep deprivation and can be used to determine the relative wakeful hours that a subject has experienced.

To quantify the activity of amylase from saliva samples, we are going to measure the rate at which substrate (starch) is reacted away. Iodine readily reacts with starch to produce a purple color. We will use an spectrophotometer to quantitatively determine the intensity of the purple color.

Before Lab:

1. Determine the final dilution ratio (e.g., 1:5; 1:10; 1:200) of salvia in the amylase reactions.

2. Determine the final concentration of starch (in units of mg) in each of the five standard curve test tubes.

3. Describe what you expect to observe for the heated saliva amylase assay.

4. Describe what you expect to observe for the unheated saliva amylase assay.

5. If no amylase activity is present in the saliva, how many mg of starch will be detected in the first time point of the assay?

Protocol

Preparing Saliva

1. Work with a partner. Collect saliva from one person in a small cup. If you haven't eaten or drunk hot liquid in a while, use your saliva. Transfer to a 1.7 mL microfuge tube and spin briefly to remove particulate matter and air bubbles.

2. Label two 1.7 mL microfuge tubes. One will be BOILED, the other kept on ICE.

3. Place 100 uL of your saliva in each of labeled 1.7 mL microfuge tube. Add 1400 uL of dH_2O. Gently invert the microfuge tubes for 2 minutes to mix.

4. Place one of the labeled tubes on ice

5. Place one of the labeled tubes in a hot water bath for 10 minutes. After the heating is complete, place the tube on ice.

Preparing a Standard Curve

1. Prepare small glass test tubes:
   Tube 1: 2950 uL dH_2O + 50 uL iodine reagent
   Tube 2: 2850 uL dH_2O + 100 uL 0.25 mg/mL starch + 50 uL iodine reagent
   Tube 3: 2650 uL dH_2O + 300 uL 0.25 mg/mL starch + 50 uL iodine reagent
   Tube 4: 2350 uL dH_2O + 600 uL 0.25 mg/mL starch + 50 uL iodine reagent
   Tube 5: 2000 uL dH_2O + 950 uL 0.25 mg/mL starch + 50 uL iodine reagent

2. Cover with parafilm and invert many times to mix.

3. Determine the final concentration of starch (units of mg) in each tube.

4. Record the absorbance of each tube at 620 nm in your lab notebook using Tube 1 as a blank. Make sure to record the absorbance of Tube 1.

Amylase Assay

1. Add 9.5 mL of 0.25 mg/mL starch solution to a 15 mL conical tube, and place the tube in a 37 C water bath.

2. Add 1950 uL dH_2O + 50 uL iodine reagent to each of 18 small glass test tubes.

3. Note the time (or start a stopwatch). Add 0.5 mL of diluted saliva solution that has not been heated to the 15 mL conical tube. Gently, invert many times to mix.

4. Immediately, remove 1 mL of reaction mixture and transfer it to one of the small glass test tubes that contains iodine solution. Record the time. Return the conical tube to the 37 C water bath.

5. Cover the time-point test tube with parafilm and invert many times to mix.

6. Record the absorbance at 620 nm.

7. After a minute has passed since the removal of the previous sample, remove 1 mL of reaction mixture and transfer it to one of the small glass test tubes. Record the time. Return the conical tube to the 37 C water bath.

8. Cover the time-point test tube with parafilm and invert many times to mix.

9. Record the absorbance at 620 nm.

10. Repeat every minute. Be careful to record times and sample readings. You may need to increase or decrease the time interval between samples depending on the activity of your saliva.

11. Repeat for the heated sample.

Data Analysis and Questions

1. Summarize your absorbance data for the standard curve in a well labeled table.

2. Use Excel to prepare a graph of $A_{620\ nm}$ vs. [Starch] (mg) for the standard solutions. Add a line to your data. Include the equation of the line on the plot. Print the polished graph and staple it in your notebook. This allows you to convert between absorbance and mg of starch.

3. Summarize your absorbance data for the amylase assays in well labeled tables. Add the zero timepoint using your value from the prelab questions and your equation from #2.

4. Make a plot of A_{620nm} vs. time in minutes for your unboiled sample. This data should be an exponential decay. If the data are a linear decay, use a line instead. Include a polished plot with equation.

5. Determine the rate of starch hydrolyses from the equation of your plot from #4. Show your work. For exponential decay:

$$A_{620} = (Amplitude)e^{-(rate)*time}$$

For linear decay:

$$A_{620} = -(rate) * time + y_{int}$$

6. The rate from #5 is in units of A_{620} per minute. Use your standard curve equation in #2 to convert the rate to units of mg of starch per minute. Show your work. Determine the standard deviation in this rate. [You would need to repeat this multiple times to get a realistic error estimate.]

7. This rate of starch hydrolysis is for the dilution that you originally created. Determine the rate of starch hydrolysis that you would expect for your full-strength saliva. Show your work.

8. Make a plot of A_{620nm} vs. time in minutes for your boiled sample. What is the purpose of this data set?