LAB

Jacques UBER
October 19, 2013

Date Performed:	October 16, 2013
Partners:	Jacques Uber
	n/a
Instructor:	Arick Rouhe

1 Introduction

A major contributer to metabolic processes are large proteins called enzymes. In cells an enzyme is usually acting as a catalyst in an aqueous solution. The substance that an enzyme acts upon is known as the substrate. An enzyme molecule latches onto one or more substrate molecules at a location on the enzyme known as the active site – this larger molecule comprised of the enzyme and the substrate is known as the enzyme-complex (Morgan and Carter, 2011).

The rate at which an enzyme converts substrate to product is influenced by three major environmental factors: the pH of the aqueous environment, the temperature of the aqueous environment, and the ratio of enzyme to substrate.

The affect of pH on enzymes is a result of the shape of the protein be influenced by either excess or lack of OH^- or H^+ ions. The pH a protein is most productive at is known as the optimum pH and every enzyme has a slightly different optimum pH.

The temperature of the environment will affect that rate at which an enzyme will do work. Most enzymes have a maximum level of efficiency where temperature increases or decreases will only start to hinder the enzymes ability to convert substrate into product. At high temperatures noncovalent forces that hold a protein's secondary and tertiary structure in place begin to break down and the protein begins to denature.

The ratio of substrate to enzyme is a factor in reaction rate as well. Most enzyme molecules react with one substrate molecule at a time, thus when every enzyme molecule becomes busy there are no free enzyme molecules to bind to additional substrate molecules. This saturation point is different for every enzyme and is a function of how fast an enzyme does its work (Biochemical and Diagnostics, 1972).

In this lab we are considering α-amylase , an enzyme that that plays a crucial role in animals as an enzyme that helps break down starch during digestion. α-Amylaseis also used in industrial processes as a starch liquefaction agent and as a detergent. Starch is made up of two polysaccharides: amylose and amylopectin. Amylose is comprised of α-glucosemonomers connected via a α1,4-glycosidic bond. Amylopectin is made of similar α-glucosemonomers but is more branched than amylose due to occasional α1,6-glycosidec bonds between two glucose. α-Amylaseis able to cleave these α1,4-glycosidic bonds.

α-Amylase, like any enzyme, is affected by pH concentration, temperature, and substrate concentration. α-amylase is active between pH 1.0 and roughly pH 11.5 which is important because industrial uses of α-amylase are usually carried out at the extreme ends of the pH scale. (Nielsen and Borchert, 2000).

Thermostability is the ability for an enzyme to experience temperature change and still function as a catalyst. α-Amylaseis able to resist denaturing at at a broad range of temperatures. α-Amylasehas been observed breaking down starch at a wide range of temperatures ($40\,^\circ$C - $100\,^\circ$C) (Fitter, 2005).

The third major influence on α-amylase is the ratio of enzyme to substrate. This is the was the focus of our hypothesis. What is the influence of α-amylase concentration on the rate of starch digestion? We proposed that the rate at which α-amylase digests starch (amylose and amylopectin) is proportional (linear) to the concentration of α-amylase present.

We will perform experimental trials that measure the time it takes for α-amylase to digest all the starch in a solution with certain α-amylase-to-starch concentration.

To make a prediction, we first define the following:

- E: An experimental trial.
- t: The time it takes for amylase to digest all the starch in a solution.
- a: The percentage of amylase in a solution.

The equation $E(a) = t$ represents the experimental trial that used a and took t time to complete.

$$\text{IF } E(a_i) = t_i \wedge E(a_j) = t_j \text{ THEN } \frac{t_i}{t_j} = \frac{a_i}{a_j}$$

TODO

2 Methods

The experiment was performed by five separate groups. Each group prepared a set of 5 test tubes containing a total of 5 mL solution; the tubes contained 1:1, 1:3, 1:7, 1:15, and 1:31 concentrations of α-amylase to water, respectively.

A second set of corresponding tubes were setup that contained 2 mL of α-amylase solution from the first set of tubes and an additional 40 drops of pH 6.8 buffer solution. These tubes were then lightly hand mixed.

An iodine (I_2KI) plate (4 wells by 6 wells) setup. Two drops of amber colored iodine solution were placed into each well.

For each test tube containing a different concentration of α-amylase the following steps were performed:

1. Mix 1 mL of 0.5% starch solution into the amylase dilution (This is time 0)
2. Place two drops of the mixed solution into the first iodine well.
    a. If the iodine well remains an amber yellow, stop the timer.
    b. If the iodine well remains a dark blue, wait 30 seconds and go to step and repeat the process using a new iodine well.

Since we know that I_2KI maintains its amber color when no starch is present and turns dark blue when starch *is* present, we can detect when the α-amylase has consumed all of the starch in a solution. Using this method we can calculate our t values for various concentrations of α-amylase .

3 Results

4 Discussion

References

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