John Sciullo
Period-4
12-09-09

AP Lab 2 Enzyme Catalysis

Abstract: AP Lab Enzymes, introduced us to the catalyzing proteins called, enzymes, focusing on the effects that various variables have on enzyme reaction rate. To further our knowledge of enzymes, we used a variety of chemicals to stimulate different conditions. Using sulfuric acid ($H_2SO_{4)}$ we were able to stimulate an acidic environment. By boiling water in one part of the experiment, we were able to see the effects of temperature on reaction rate. Overall we tested a wide array of variables, investigating their effects on reaction rate. At the end of the experiment we were able to come up with the following viable conclusion; the variables that had the most significant impact on reaction rate had to do with changes in pH levels of the solutions, and the temperature.

Splitting off into groups we began the lab by observing a reaction in which catalase was placed into a solution, releasing bubbles of O_2. This reaction introduced us to enzymes and their activities. Shortly after, we began investigating the effects various variables had on the reaction rate. In demonstrating the effect of boiling on enzymatic activity, we were able to learn that sharply increasing the temperature of the enzymatic solution, the reaction rate lowered. As a group, we inferred that the high temperatures caused the enzymes, proteins, to unravel and denature. We came to the same conclusion upon testing the effects of pH.

The last sections of the lab called upon us to investigate the differences between the uncatalysed rate of H_2O_2 decomposition and the enzyme-catalyzed rate of H_2O_2 decomposition. Investigating the uncatalyzed rate of decomposition, an individual was picked from the group to come in the following day and test the effect of leaving the H_2O_2 in room temperature for 24 hours. By comparing the results between the two, we were better able to understand the effects enzymes play in reactions.

Emphasis: Data Analysis and Collection

Objectives: Before doing this lab you should understand:

- the general functions and activities of enzymes;
- the relationship between the structure and function of enzymes;

- the concept of initial reaction rates of enzymes;
- how the concept of free energy relates to enzyme activity;
- that changes in temperature, pH, enzyme concentration and substrate concentration can affect the initial reaction rates of enzyme-catalyzed reactions; and
- catalyst, catalysis, and catalase

After doing this lab you should be able to:

- measure the effects of changes in temperature, pH, enzyme concentration, and substrate concentration on reaction rates of an enzyme-catalyzed reaction in a controlled experiment; and
- Explain how environmental factors affect the rate of enzyme-catalyzed reactions.

Problem: What kind of roles the factors of pH levels and temperature have on various reaction rates. What roles inhibitors and activations play on enzymatic reactions.

Observations:

- **Enzymes** are proteins produced by living cells; they act as catalysts in biochemical reactions. A **catalyst** affects the rate of a chemical reaction. One consequence of enzyme activity is that cells can carry out complex chemical activities at relatively low temperatures.
- In an enzyme-catalyzed reaction, the substance to be acted upon, the **substrate**, binds reversibly to the active site of the enzyme.
- The **active site** is the portion of the enzyme that interacts with the substrate, so that any substance that blocks or changes the shape of the active site affects activity of that enzyme.
- **Salt concentration** plays a huge role on the effectiveness of enzymes. If the slat concentration is close to zero, the enzyme will denature and form and inactive precipitate. If the salt concentration is very high, normal interaction will be blocked.
- **pH**, as the pH is lowered, an enzyme will tend to gain H+ ions and eventually enough side chains will be affected so that the enzyme's shape is disrupted. Likewise, as the pH is raised, the enzyme will lose H+ ions and eventually lose its active shape.
- **Temperature**, usually chemical reactions speedups the temperature is raised. As temp increases, molecules have more kinetic energy. If keeps increasing, a **temperature**

optimum is reached. Kinetic energy of the enzyme is so great that the conformation of the enzyme molecules is disrupted.

- **Activations and Inhibitors**. If a molecule increases the rate of the reaction it is an **activator**, and if it decreases the reaction rate it is an **inhibitor**.

Data: **Page 23: Exercise 2A: Test of Catalase Activity**

1.
   a. What is the enzyme in this reaction?
      Catalase solution
   b. What is the substrate in this reaction?
      H202 (Hydrogen Peroxide)
   c. What is the product in this reaction?
      Water and Oxygen
   d. Through the usage of a wooden splint

2. How does the reaction compare to the one using the not boiled catalase? Explain the reason for this difference.

   In this reaction no bubbles formed. The catalase did not work because the high temperature denatured it.

3. What do you observe? What do you think would happen if the potato or liver was boiled before being added to the H2O2?

   Many bubbles formed. If the potato or liver was boiled before being added no reaction would form since the catalase in the potato or liver would be denatured.

Exercise 2B: The Base Line Essay

Base line (Final – Initial) - **76 mL** KMnO4

Exercise 2C: The Uncatalyzed Rate of H2O2 Decomposition

Base line of un-catalyzed solution – **112 mL**

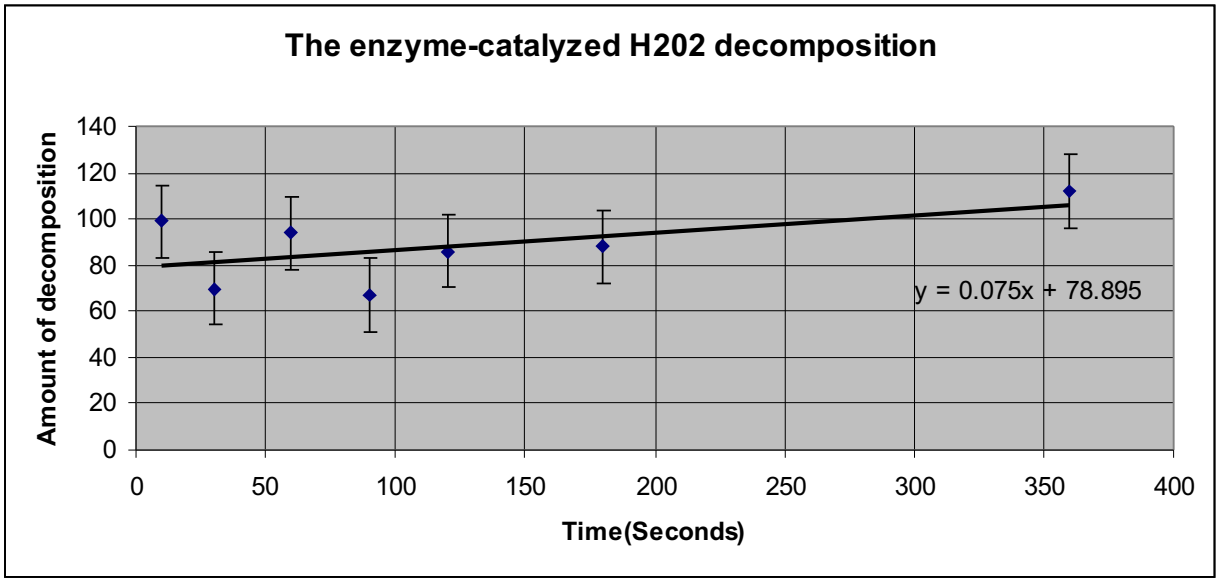
Exercise 2D: An Enzyme-Catalyzed Rate of H2O2 Decomposition

Base line (Final – Initial) **105 mL** KMnO4

Procedure for a Time-Course Determination

KMnO4	Time (seconds) 10	30	60	90	120	180	360	Uncertainty (Standard Deviation)
a) Base Line	106	106	106	106	106	106	106	0
d) Amount of KMnO4 consumed	99	70	94	67	86	88	112	15.821926
e) Amount of H2O2 used	7	36	12	39	20	18	-6	15.821926

Independent Variable: Time in Seconds
Dependent Variable: Amount of H2O2 used (A



Analysis of the Results

1. From the formula described earlier, recall the rate= change in y/ change in x.
   Determine the initial rate of the reaction and the rates between each of the time points.

	Time Intervals (seconds) Initial 0 to 10	10 to 30	30 to 60	60 to 90	90 to 120	120 to 180	1800 to 360
Rates	0.10	-0.68	1.25	-0.9	.63	0.03	0.13

2. When is the rate the highest? Explain why.
   The rate of the reaction is fastest from 30 to 60 seconds that is when H2O2 is decomposed at the fastest rate.

3. When is the rate the lowest? For what reasons is the rate low?
   The rate is lowest from 60 to 90 seconds. This is because the amount of swirling acts as an inhibitor, slowing the rate of decomposition of the H2O2.

4. Explain the inhibiting effect of sulfuric acid on the function of catalase. Relate this to enzyme structure and chemistry.
   Sulfuric acid, H2SO4, greatly reduces the pH level of the catalase solution, causing it to become acidic. The acidity of the solution begins to denature the catalase and subsequently inhibit its function. This is seen as it takes more drops to produce a reaction.

5. Predict the effect that lowering the temperature would have on the rate of enzyme activity. Explain your prediction.
   Lower the temperature would have profound effects on the rate of enzyme activity. Lowering the temperature, the rate of the reaction would slow, as temperature, along with pH and salt concentration, is a factor that can cause changes in rate of activity. Lowering the temp, would greatly decrease the rate of the enzyme activity.

6. Design a controlled experiment to test the effect of varying pH, temperature, or enzyme concentration.
   A good experiment to test the varying effects of pH, temperature, and salt concentration on proteins can be just like the one outlined in the lab manual. Splitting the experiment into three different parts, students would be able to individually see the different effects various variables have on the rate of enzyme activity. Testing temperature, students should investigate the rate of reaction at three distinct temperatures; 0**°C**, 40**°C** and 80**°C**. Comparing the different rates, students would be able to come up with a conclusion on the effects of temperature. Salt concentration can be investigated in a very similar fashion. Students, given three different solutions, each with varying salt concentrations (0 M, 2 M, 5 M) would be able to conclude the effects salt concentration has on enzyme activity. pH can also be tested in similar fashion as the two previous variables. Having three different solutions differing in acidity/alkalinity, students would be able to clearly see the effects of varying pH levels.

Conclusion:

In completing this lab, the problem posed earlier can be easily answered. Investigating the significance of various variables to the rates of the reaction, it is clear that the both variables mentioned in the problem, pH level, and temperature have profound effects on the rates of enzymatic reactions.

Working with sulfuric acid, we were able to see the effects that increased acidity had on an enzymatic reaction. Increased acidity level of a solution, or drastic changes in pH for that matter, plays an important role in determining the rate of an enzymatic reaction. The more drastic change in pH, in other words the more acid/alkaline the solution, the higher chance of a lower rate of reaction. This phenomenon is due to the nature of proteins and can be clearly seen in Exercise 2A of the lab. Although our data is slightly flawed in that it appears that in the reaction the solution needed fewer drops of KMnO4 to perform the reaction. Through deductive reasoning as well as further research we are able to reinforce our conclusion that low pH denatures protein enzymes causing reaction rates to slow down. Adding further support to the conclusion, the general procedure states that before all of the H2O2 is converted to H2O and O2, the reaction is stopped by the sulfuric acid.

Investigating the effects of temperature proved to be easy as well. The effect temperature plays on reaction rates can clearly be seen in the experiment preformed in Exercise 2S. Demonstrating the effect of boiling on enzymatic activity, we transferred 5 mL of purified catalase extract to a test tube and placed it in boiling water. Comparing the results to the ones gathered using the un-boiled catalase; we can see that there is a clear difference. Adding 1 mL of the unboiled catalase, the reaction occurs at a much quicker pace than the reaction that occurred when the boiled catalase was used. Although we do not have quantitative data to back up these results, the qualitative data gathered fully supports are argument. Furthermore, the background data presented in the beginning sections of the lab strengthens our argument. In completing this part of the lab, we are able to conclude temperature plays important roles in determining the rates of reactions. Despite the inaccuracies displayed by our data, the conclusions we have reached are viable and explainable fully answering the presented problems.

Discussion:

Provided at the start of the lab were several pages of background information that proved to be highly useful during the lab. The information defined enzymes and thoroughly described their general functions and activities. In general, enzymes are proteins produced by living cells; they act as catalysts in biochemical reactions, meaning they affect the rate of chemical reactions. Enzymatic reactions and the rates at which they occur are studied thoroughly throughout the lab. The relationship between the structure and function of enzymes, poses an interesting question (the problem), guiding us through the lab.

Posing the question of how the variables of pH level in solution and temperature affect the rate of reaction, the relationship between structure and function is further developed. By the end of the lab, it was pretty clear that there was a very strong relationship between the two, as the changes in reaction due to the variables were attributed to the structure of the enzyme, a type of protein. This relationship was directly tied to the concept of free energy. As the variables influenced the reaction rates, the amounts of energy catalyzed by the enzyme fluctuated. As the enzyme denatured, the reaction rate grew smaller and smaller, as the enzyme was no longer able to function properly.

The conclusion given outlines the effects that changes in temperature and pH play on the initial reaction rates. The more drastic the temperature, the higher the initial reaction rate, as the enzyme has denatured. pH affects initial reaction in the same fashion. The greater the change in pH, the higher the reaction rate, as the enzyme has denatured. After finishing the lab, we were able to measure the effects of changes in temperature and pH. As the temperature increased in the enzyme, the reaction rate grew slower and slower. The same relationship was seen in pH levels. As the pH changes, the reaction rate grows slower and slower.

In following the procedure outlined in the AP lab manual, there were no problems stemming from the procedure itself that would have potentially challenged the results gathered. Whatever inaccuracies the results present were due to the carelessness of those involved in collected them. Throughout the lab, there was only one problem experienced. The problem stemmed from the inability to add single drops of $KMnO_4$ to the solution, as the pipettes proved to be hard to handle. Several times when adding the chemical to our mixture, instead of a drop, a small stream

would be released by the pipette. Although this problem proved to be a hindrance when it came time to gather quantitative data, it does not disqualify or challenge our conclusion. The quantitative data gathered as well as the countless explanations and theories backing it up, leave the validity of our conclusion in no doubt.

Along with the slight problem, the one limitation we encountered dealt with the absence of barometers. Instead of using barometers, as outlined in the procedure, we used a slightly less accurate way of attaining baseline. This limitation did nothing to invalidate the gathered results, as it only influenced the quantitative data gathered, as opposed to quantitative.

A weakness in the procedure also has to be addressed. Although the procedure outlines a practical way of investigating the problem, it leaves to many variables open to the environment. Having a lack of control variables, the lab remains subject to the countless ever-changing variables. A person performing this experiment in the Arctic will attain tremendously different results than one performing it in the Mojave Desert.

Fortunately solutions and simple improvements exist to better the procedure and rid it of the problems, limitations, and weaknesses outlined previously. A simple solution to the pipette problem would be to acquire and use droppers. In doing so we would be provided accurate, viable quantitative data that would support our ultimate conclusion. Had we been supplied droppers, we would have been able to add a further dimension to our conclusion. A similar solution can be used to solve the problem of a lack of barometers. Although we found a way to bypass the need for one, it was a minor inconvenience and caused us to make some changes to the procedure. However, our conclusion would in no way change had we been given access to barometers. Lastly, to rid the lab of any weaknesses, the procedure would have to be changed to access and outline the wide array of variables that must be controlled to assure the validity of the data.

The skills and knowledge gained from this lab enabled us to better understand life's biological processes. The knowledge we gained can be applied to real life situations, and often is, in the case of biologists and other professionals in the scientific field. A pharmacist trying to achieve a reaction needs to have a deep understanding of the workings of enzymes and other catalysts. The

importance of enzymes can be seen throughout the world, without them, life as we know it would not exist.