Investigating the Effect of Ph on the Activity of the Enzyme Catalase

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Introduction

Hydrogen peroxide (H2O2) is a very pale blue liquid that appears colorless in a dilute solution, slightly more viscous than water. It is a weak acid. It has strong oxidizing properties and is therefore a powerful bleaching agent that is mostly used for bleaching paper. Catalase is a common enzyme found in all living organisms. Its functions include the conversion of Hydrogen Peroxide, a powerful and potentially harmful oxidizing agent, to water and oxygen. One molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second. The liver and other living tissues contain the enzyme catalase. Hydrogen peroxide, which is a harmful by-product of the process of cellular respiration is broken down if it builds up in concentration in the cells. If we use potato or other tissue containing this enzyme, we can use this to measure the relative influence of varying different factors on the activity of enzymes in living tissue, the factor I will be investigating in my coursework is the activity of pH.

Aim

The aim of my investigation is to find out how different pH's will affect the enzyme activity and how this will affect the rate of reaction. Extremely high or low pH values generally result in complete loss of activity for most enzymes. pH is also a factor in the stability of enzymes.nAs with activity, for each enzyme, there is also a region of pH optimal stability. I will also be measuring the rate at which oxygen is evolved and how it reflects the activity of the enzyme catalase. Enzymes are generally globular proteins that have primary, secondary, tertiary, and maybe quaternary structures. They are biological catalysts that can speed up a reaction rapidly. Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge, and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity therefore only one substrate will fit into the active site of the 1

enzyme. Two theories on Enzyme function: Lock and Key hypothesis - where you have 1 enzyme, 1 substrate and there are a complementary shape and charge.

Induced Fit Hypothesis

The substrate and enzyme are not complimentary. During a collision, the substrate induces a change in the active site shape and so it becomes complimentary. E. g. Hand in Glove. Some enzymes are produced in an inactive form, therefore, need to be switched on by the addition of a non-protein group, this sometimes causes a permanent change.

Limiting factors. Few factors that affect enzyme activity.

Temperature Each enzyme has an optimum temperature at which it works best. A higher temperature generally results in an increase in enzyme activity. As the temperature increases, molecular motion increases resulting in more molecular collisions. If, however, the temperature rises above a certain point, the heat will denature the enzyme, causing it to lose its three-dimensional functional shape by denaturing its hydrogen bonds. Cold temperature, on the other hand, slows down enzyme activity by decreasing molecular motion.

The rate of reaction is slow due to insufficient energy (kinetic), this means there are very few collisions between substrate and enzymes.
Rate of reaction increases due to more kinetic energy and more collisions. More enzyme-substrate complexes are formed.
It has now reached an optimum temperature where there's optimum energy. There are many successful collisions therefore more product has been formed.
The rate of reaction starts to decrease, The increase in kinetic energy now causes molecules within the enzyme to vibrate. This may result in the hydrogen bonds breaking therefore enzyme structure may change,. All the enzymes have still not been affected at this point and there has not been a permanent change.
An increase in energy causes molecules to vibrate. 4 bonds (S-S, ionic bonds) begin to break. The tertiary structure is now permanently altered and therefore the active site and substrate are no longer complimentary so no products can now be made. PH Each enzyme has an optimal pH that helps maintain its three-dimensional shape. Changes in pH may denature enzymes by altering the enzyme's charge. This alters the ionic bonds of the enzyme that contribute to its functional shape. Most human enzymes work at an optimum pH of around 7. 3 or 7. 4. The majority of these are intracellular enzymes. Extra-cellular enzymes e. g. digestive enzymes work at more extreme pH's e. g. protease at pH2 and arginase at pH 11. The rate of reaction is affected by changes in pH. The charges on R groups maintaining a structure of the active site are essential for the formation of the E-S complex.

If the properties of the active site change, the substrate can no longer combine (I. e. increase in protons of hydroxyl ions will repel the substrate) pH can also affect individual amino acids as the formation of zwitterions are common. Enzyme Concentration Assuming a sufficient concentration of substrate is available, increasing enzyme concentration will increase the enzyme reaction rate. If you increase the enzyme concentration there is more chance of a collision and an increase in the rate of reaction. When there's more substrate than enzymes (substrate becomes the limiting factor) it will stay at one level. Substrate Concentration Rate of reaction will increase proportionally to the sub concentration until V-max. V-max is where all the enzymes are occupied in E-S complexes. This is where the reaction will stay at one level.

At a constant enzyme concentration and at lower concentrations of substrates, the substrate concentration is the limiting factor. As the substrate concentration increases, the Enzyme reaction rate increases. However, at very high substrate concentrations, the enzymes become saturated with substrate and a higher concentration of substrate does not increase the reaction rate. Validity and Reliability As I've just discussed, various factors affect enzyme activity. For this piece of coursework, I will be concentrating on changing just 1 factor (pH) to show that this is the one that can change results. This will help me work out close trends and get me a good range of results. I am going to show this by doing an experiment with potato disks. I will be using different pH buffers in my experiment; from pH3 to pH9, and will have a range of variables; dependant and independent. My dependant variable will be the time it takes for the red dye to move across the monometer. I will be keeping the temperature the same as it is difficult to control and is a limiting factor, and so to record results and minimize fluctuations I will do the experiment at the same time of day. I will measure temperature as I know it will affect enzymes and the rate of reaction. I will be using the same concentration and volume of hydrogen peroxide (substrate) because if I change too many variables it will make it difficult to know which affected the results. I will also be using the same number of potatoes, same variety of potatoes, same mass of potatoes, to indicate I am using the same enzyme concentration. All these points will make my results valid but to increase reliability I will also do the experiment 3 times to eliminate anomalous results. Also as I am using pH 3 to pH 9 I am going from acid to alkali giving me a wide range of results. I will wash my equipment each time to increase reliability and I will also use the exact same equipment to make my results constant. Safety Hydrogen peroxide is an irritant and highly corrosive, therefore. I will have to wear safety goggles to protect my eyes. I must wash it off my skin. It will bleach clothes.

Method

I will collect all the equipment as shown in the apparatus list and then set them up.

I will then take a reading from the thermometer of the water. This will be my accountable variable.
Then, I will cut out a potato cylinder by using a cork borer. I will push the cylinder of potato out by the small cork borer.
On the white tile, I will place the potato cylinders. Then by using a scalpel, I will cut out the potato pieces into small disks, all equal sizes, and then I will measure the mass of them by using a balance and I will average it out to 1g.
Then I will inject the red dye into the monometer by using a clean syringe and make sure there are no bubbles in the monometer. I will take the reading of where the red dye is.
I will then clamp the monometer onto the stand so it is upright and stable.
Then, by using a pipette, I will pour 10cm? of hydrogen peroxide into one of the measuring cylinders. Then, by using another pipette, I will pour 10cm? of pH buffer solution and pour it into the other measuring cylinder. I will keep this volume the same for each of my tests to make it fair.
I will then pour the potato disks and the buffer into the test tube. I will place this into the conical flask and will record the point at which the red dye is at on the monometer.
Then, I will connect the test tube to the monometer by the delivery tube.
I will then place the hydrogen peroxide and straight away after this, I will place the bung on top of the test tube straight away to stop oxygen from escaping. I will clip on the clip to one of the delivery tubes and close it. We put the products in this order because the reaction starts as soon as the hydrogen peroxide goes in and we want the reaction to start just before we put the bung on so none of the oxygen escapes.
I will start the stopwatch after this.
I will measure how much the dye moves in 1 minute.
I will measure the distance moved and will take an average for the pH.
I will repeat the experiment form step 2 for each pH 3 times to make it reliable.

There are relationships between the amount of oxygen produced and the pH used. Also, between the pH and the rate of reaction. The starting parts of both graphs shows a positive gradient, this reflects the general effect of increasing pH on the rate of reaction. It shows that there is a positive relationship between increasing the pH from 3 to 6 and the rate of reaction and the amount of oxygen produced. There is a steady increase from pH 3 to pH 6 and it increases, there is more distance that the dye has moved and therefore this shows an increase in the rate of reaction. At pH3, it moves 1.7mm in one minute. But as the pH number was increased to pH4, it had increased to 4. 7mm. This is an increase of 3. 0mm. But, then there was only a small steady increase at Ph5 to 5. 7mm, only an increase of 1mm. Then at pH6, there was another increase to 8mm which is an increase of 2. mm. This shows that the biggest increase was from pH 3 to 4. It can be seen from the graph that at pH 5 and pH 6, the dot was not on the curve of best fit proving it to be an anomalous result. But

overall this proves that there is a positive correlation between the increase in pH and the rate of reaction. After pH 6, it was increased to pH 7, where there was the greatest rate of reaction. It can be seen from the graph that at pH 7, it was the highest point proving it to be the optimum pH.

It moved the red dye 11. 7mm in one minute which was the highest amount that it had to move out of all the PHs.It had increased 2. 7mm. The peak on the graph provides evidence that the rate of reaction is at its maximum and the enzyme catalase works best at pH 7. Then after pH 7, the descending part of the graph shows that there is a loss of catalase activity taking place at pH 8 and 9. There is a slow decrease after pH 7 and it is a negative correlation. The gradient is similar to the positive gradient of Ph 5 and 6. From pH 7 to 8, there is a decrease of 2. 4mm, from 11. 7mm to 9. 3mm. From pH 8 to 9, there was a steady decrease of 4. 0mm, from 9.3mm to 5. 3mm.This in turn shows that there is a negative correlation and therefore showing that the rate of reaction is decreasing. The curve of best fit shows that there are two anomalous results at pH 5 and pH 6 therefore all the other points are correct. There is a positive gradient until pH 7 which is the optimum pH and then after this, there is a negative gradient. This means that there is a relationship between the pH and the rate of reaction. The PH's before the optimum are all acids and it shows the rate of reaction for them to be increasing.

This shows that the more acidic it is, the lower the rate of reaction. After the optimum ph, there is a decreasing gradient showing the more alkaline the solution is, the lower the rate of reaction. In conclusion, I can say that as the pH increases, so does the rate of reaction meaning that the enzyme activity increases. Then at pH7, the enzyme activity is at its maximum and so is the rate of reaction. After Ph 7, as the pH increased, the rate of reaction decreased. I have made this conclusion by the results that were collected, my graph, and my scientific knowledge shown below. As the pH became more alkaline the rate of reaction decreased. Also, as the pH decreased to pH 3 and 4 (acid), the rate of reaction was slow. This occurred as the charges holding the tertiary structure together would have been affected. Also, the changes in the active site would be damaged. As there is an increase in H+ they bind with the negative charges in the active site, stopping the substrate binding with the enzyme. When the charges change, the enzyme is permanently destroyed and cannot work, meaning it is denatured. This means less enzyme-substrate complexes form when pH is acidic, leading to fewer products being formed. PH 3 moved the red dye 1.mm; PH4 moved it 4. 7mm in one minute. At pH 5 the rate of reaction increases to 5. 7mm. After this point, there is only a slight increase (at pH 6). The rate of reaction is lower than that of pH 7 as they are more acidic but do not contain as many H+ ions as pH3 and pH4. However, the active site of the enzyme is still affected and causes the charges to become damaged leading to denatured enzymes.

Although not as many enzymes become denatured leading to a slightly higher number of products being formed (not as great as pH7). PH 5 moved the dye 5.

7mm whereas pH6 moved it 8. 0mm in one minute. The rate of reaction at pH 7 is the greatest as there are a great number of enzyme-substrate complexes being formed. This is the best pH for the enzyme to collide with the substrate and successfully bind. The charges holding the tertiary structure and the enzyme and substrate together are unaffectedly leading to a higher amount of product being formed. It moved the red dye 11. 7mm in one minute. As the pH became more alkaline the rate of reaction decreased. At pH8 the rate of reaction decreased and at pH 9 it decreased even more. This is due to an increased number of OH- ions. These have the same effect as H+ ions and damage the active site. The OH- ions bind with the positive charges in the enzyme and active site. Without the charges present the substrate no longer fits, resulting in the enzyme being denatured. This leads to less enzyme-substrate complexes, and a lower amount of product being formed. Therefore the results from the investigation and the graphs show that the optimum pH is pH7. This is due to the fact that this pH does not affect the active site. The catalase and hydrogen peroxide bond form water and oxygen successfully. PH 7 allows the reaction to take place faster as enzyme-substrate complexes can be formed to produce a product. Also, the more acidic or alkaline the pH became, the more the rate of reaction decreased. This occurred as it affects the bonds in the active site of catalase. The catalase is no longer able to function as it is denatured. Fewer enzyme-substrate complexes are formed and the blue dye only moves a small distance.

EVALUATION

During my investigation, I recognized that there were limitations to both the apparatus and techniques used. Even though I tried my best to keep all variables constant and made an effort to reduce problems, not all could be kept constant. Firstly, there were difficulties with handling various apparatus which restricted the reliability of my experiment. The manometer was used to find how much oxygen was produced. This was the better device to use rather than the gas syringe as it contained the best way of getting measurements. This made my results more accurate. However, this device also contains a couple of limitations. The monometer appeared quite simple to use and I thought that it would be easy But it turned out this was the hardest piece of equipment instead and caused me the most problems. When the red dye was inserted into the odometer, air bubbles were frequently produced and this would have affected my results if I didn’t correct it. This was a continuous problem that caused many difficulties. Another problem was bungs.

This was used to keep the test tubes blocked to stop the oxygen escaping. One of the tubes had to be completely blocked using a clamp so no oxygen could escape; this had to be done a couple of seconds after the catalase was added. This was difficult to do and was not done as soon as the reaction began. This would have resulted in some oxygen escaping giving me a slightly lower number of oxygen produced than was actually produced. The bung also had to be placed seconds after

the catalase was added, this could have also resulted in some oxygen escaping. There could have been problems also because of my technique. This is because I used potatoes as the form of my catalase. But, there could have been different concentrations of catalase present in the different discs. The temperature was kept constant using a water bath, the temperature of this was 25? C (room temperature). However, there is one limitation as the investigation was carried out over a period of a couple of days. The room temperature may have had an effect, as the temperature is not constant over a long period. An increase in temperature would give the particles higher energy and so there would be an increase in collisions. The effect of sunlight on hydrogen peroxide might have had a problem on the stability of it as it was left out of the brown bottle that would normally protect the chemical. However, the limitations mentioned above did not greatly affect the reliability of my results.

It had a minimal effect as the results are reliable and also the curve of best fit is reliable. Everything in my experiment happened the way it should have happened but there were two anomalous results at pH 5 and pH6. But, this wasn't that unreliable because I think that this happened because of the change in my temperature. At this pH, the temperatures changed from 25 C to 26 C. There was a slightly higher rate of reaction than what it should have been according to the curve of best fit but it still follows the pattern that there is a positive gradient for the acids and a negative gradient for the alkalis. My conclusion that I came up with was that as pH increased, the rate of reaction of the enzyme increased, and then at pH 7 it reached its maximum, and as the pH's increased after, the rate of reaction decreased. I think that this conclusion is fairly accurate. This is because my graph showed exactly this because there was a positive gradient for the acids and at pH7 it reached its maximum, and then there was a negative gradient for the alkalis. But, there were the two anomalies but this was because of a variable. In conclusion, I believe that my conclusion is fairly reliable even though there were a few limitations and problems. This is because the problems that have existed have not had a significant effect on my results and my conclusions prove the aim.