An investigation into the effect of varying ph on enzyme activity essay sample

Literature, Russian Literature

Protease is an enzyme that reacts with protein to break it down into its constituents. Enzyme activity can be affected by the change of pH, temperature and the concentration of substrate or enzyme in the solution. The focal aim of this experiment is to see what pH our protease works at best to determine where it is made in the body. The casein used comes from marvel milk and is a protein. It is a protein that is present in milk to give it its white colour. When broken down into amino acids, the milk becomes clearer.

When the protease reacts with the casein, it turns clearer from its original cloudy colour. The reaction therefore would be monitored using a colorimeter that measures the amount of light going through the solution and the more light that is let through, the more the amount of substrate has been broken down. However the colorimeter uses a filter to only let some wavelengths of light through or else it would let all the light through not showing distinctions between different solutions.

Therefore a filter must be used and as the come in different colours letting only some wavelengths of light through so the most suitable was chosen for the experiment which was 490 nm. (A filter was not necessary for the experiment but one had to be used as the colorimeter would not work without one.) The buffer solutions are solutions that resist changes in their pH, even when small amounts of acid or base are added.

They are said to be amphoteric. So if 8. 8 pH buffer solution is added, it would mean that the total pH of the solution is 8. and they keep the pH constant but if the solution gets acidic to the pH of 8. 9, it would remove some hydroxide ions to get the pH back to 8. 8. Method All of the testubes

will be washed thoroughly and rinsed out with distilled water to minimise the chance of contamination that could affect our results. There are several factors that could affect our rate of reaction. A rise of temperature could cause an increase in the rate of reaction as it is known this from previous experiments and scientific knowledge. This would mean keeping the temperature constant.

This may prove difficult but the experiment will be done at room temperature on the same day to ensure a minimum change in temperature. Temperature increases the rate of reaction as there is more kinetic energy given to the enzyme so it moves around faster increasing the chance of a successful enzyme-substrate collision. (Q10’s law states as the temperature goes up by 10i?? C the rate of reaction will double). Using a water bath would keep the temperature constant but very difficult as keeping the temperature constant would mean keeping the solution in the water bath until the reaction is complete.

However, this would mean that the colorimeter could not be used as it does not go into the water bath. The concentration of the enzyme and substrate would also affect the rate of reaction as higher concentrations would mean the more chance of an enzyme-substrate complex to be formed. The concentrations of enzyme and protease will therefore be kept constant. The volumes of the protease, casein (marvel milk is used in the experiment. It is a powdered milk where water can be added to turn it into milk).

The volume of the buffer solution will also be kept constant to provide reliable results. The concentration of the protease throughout the experiment will be 2% and the marvel milk 4%. A 10 cm3 measuring cylinder will be used and 2 cm3 each of casein and buffer solution will be added together in the same testube but keeping the enzyme away from it so it will not react with the casein. 2 cm3 protease will also be used and all these volumes will be kept consistent throughout the experiment.

The stop clock will be started as soon as the two solutions are mixed together in the testube and the solution in the testube will be poured into the cuvette which will be put into the colorimeter. The colorimeter has two knobs which can be adjusted finely to make the colorimeter read 100% transmission before the cuvettes containing the solutions are added this will be done by using the control prepared at the beginning containing protease and the milk letting it go as clear as possible (it will be left for 10 minutes to make sure it will go as clear as possible).

Then it will be inserted in the colorimeter and as mentioned before, the colorimeter will be calibrated to ensure it reads 100% When the solution in the cuvette goes less cloudy (More light getting let through the solution) the stop clock will be stopped and it will be measured how long it takes for the reaction to stop where the light getting through matches that of the control. This is the dependant as it is measuring the time taken for the solution to get clear (100% transmission). The manipulated variable in this experiment is the pH where buffer solution is used to alter it.

The buffer solution keeps the pH constant as mentioned in the trial experiment. The buffer solution that will be used (therefore the different pHs) will be the pHs of 4. 5, 5. 9, 7. 0, 8. 0 and 8. 8. However extremely alkaline or acidic buffers are not used as the rate of reaction would decrease as the too many H+ ions (in acidic solution or OH- ions (in alkaline solutions) would disrupt the tertiary structure of the enzymes and denature it changing the shape of the active site so the substrate will not fit any more so only slightly acidic and alkaline conditions are used.

Each different experiment of the different pHs will be repeated three times and each time the colorimeter will be checked that it reads 100% transmission or be calibrated using the control as it is highly sensitive. The results will be recorded in a table where a rate will be calculated (1 divided by time taken) for each experiment and an average will be taken to eliminate any anomalies. Any significant anomalies will mean that the experiment for that buffer solution will be redone and the anomaly will not be used to work out the average.

Apparatus Two 10 cm3 measuring cylinders- This cylinder is big enough to hold the buffer solution and the milk and the second measuring cylinder is to hold the protease. These will be washed out with distilled water before each repeat and experiment to minimise the chance of contamination Test tubes- This is where the buffer solution containing the milk will be mixed with the protease. The test tubes will be cleaned out using distilled water before each repeat and experiment again to minimise the marginal error

Test tube rack- This is to hold the test tubes so one hand can add the buffer and the marvel milk (protein) to the test tube and the other hand can add the protease. Cuvettes- These are special apparatus for the colorimeter where the solution will be added from the test tube and the percentage transmission will be measured. Colorimeter- The colorimeter comes with different filters that let different lengths of light through and measure the clearness of a solution. Water would have 100% transmission so water will be used throughout the experiment to make sure that the colorimeter show 100%.

It has two knobs; one for coarse adjustment and one for fine adjustment so when water is used we can adjust the knobs to point to 100% throughout the experiment. Distilled Water- This will be used to clear out all the apparatus and it will minimise the chance of error as it contains no decontaminants. Buffer solutions- Pre-prepared buffer solutions will be used to keep the pH constant for each different pH. Marvel milk- This marvel milk contains the casein and will be the substrate and has the concentration of 4%

Protease- This is the enzyme that will break down the casein and the volume of it used will be kept constant throughout. It has the concentration of 2% Safety There are some safety issues that need to be taken into account for this experiment. A lab coat will be worn to ensure another extra layer of protection against the buffer solutions which are alkaline or acidic so they are harmful and an irritant that may cause blistering to living tissue so safety glasses will also be worn. These ensure that any spillage will not go into the eyes.

Any spillages will be mopped up and any broken glass reported and cleared up carefully and disposed in a special glass bin. Prediction The aim of the experiment is to find out where the enzyme is made in the body. If it is found that the enzyme works best at alkaline conditions, it will determine the enzyme is made in the duodenum as from previous scientific knowledge, the duodenum has a pH of 8 that neutralises the acidity of the stomach. The enzyme in the duodenum is Trypsin. The duodenum produces sodium hydrogencarbonate that neutralises the hydrochloric acid from the stomach giving it its alkaline environment.

The pepsin in the stomach is a protease which works best at the acidic conditions of the stomach so if the rate of reaction increases in acidic conditions, it will determine the acid is made in the stomach and in the duodenum if it works best in alkaline conditions. Results In the experiment, three repeats were done for each different buffer solution. They were written down and the average time was found out. The rate of reaction was also found by dividing 1 by the average time taken and then multiplied it by 1000 to make it easier to plot on a graph.

Analysis Looking at the graph very strong positive correlation can be seen showing that as the pH increased, the rate of reaction increased (The positive correlation is represented with a red oval). The graph also shows that the enzyme used has the optimum pH of 8. 8 as the rate of reaction is the highest (58. 8). An enzyme is a globular protein molecule which in a biochemical reaction as a catalyst. Enzymes can be intracellular (work within

a cell) or extra cellular (work outside a cell). The enzyme used here was trypsin.

This conclusion was reached using scientific knowledge and research. Enzymes are very sensitive to pH changes and they all have an optimum pH where they work best at. The enzyme used in this experiment worked best at pH 8. 8 showing that its optimum pH was alkaline suggesting firstly that it was trypsin. Looking at the graph it can be also deduced that the trypsin did not work best at the acidic conditions. This suggests that if extreme pHs were to be used (lesser the pH of 5. 9) the trypsin would not work.

This is due to there being many free H+ ions (in too acidic solutions) and OH- ions (in too alkaline solutions) thus disrupting the ionic bonds, that maintain the tertiary structure and denaturing the enzyme and changing the shape of the active site therefore no reaction happening as the substrate will not fit as readily into the active site. In this investigation, the trypsin did not work best at acidic conditions and the rate of reaction was affected because active site (where the reaction happens on the enzyme) is very sensitive to pH changes away from its optimum.

This is due to the changes in the pH which upset the delicate chemical arrangement at the active site and so stopped the enzyme working efficiently. As mentioned before, this was due to too many H+ ions which altered the ionic bonds that help to determine the 3-D shape of the trypsin. This can lead to altered protein recognition or an enzyme might become inactive. Changes in pH may not have only affected the shape of an enzyme

but it may also change the shape or charge properties of the substrate so that either the substrate could not bind to the active site or it could not undergo catalysis.

To go into more detail one has to understand the structure of an enzyme and the denaturing process. The denaturing process * Enzymes are made out of proteins * Proteins are made out of amino acids. Each amino acid has an amino group (NH2) and a carboxylic acid group (COOH which can absorb a proton to change the tertiary structure). The R group is a different molecule in different amino acids where different bases can attach which can make them neutral, acidic, alkaline, aromatic (has a benzene ring structure) or sulphur containing.

The protein is very specific as mentioned before so the trypsin's primary structure is important. It depends on the order and number of amino acids in a particular protein. * The secondary structure of a protein could take on two different forms. These could be a helical shape (DNA) or a beta-pleated sheet. The beta-pleated sheets are composed of chains which are side by side and are connected by Hydrogen bonds. All the peptide linkages are involved in inter-chain Hydrogen bonding so the structure is very stable. * The tertiary structure is what makes up the trypsin into its globular shape.

The shape of the trypsin molecule is held together by Hydrogen bonds between some of the R groups and ionic bonds between positively and negatively charged R groups. The ionic bonds are weak interactions, but together they help give the protein a stable shape. The trypsin may be

reinforced by strong covalent bonds called disulphide bridges which form between two amino acids with sulphur groups on their R groups. The trypsin is a enzyme so referred to as a globular protein and consists of chains that are folded over to make a three-dimensional shape and give its specific shape (which in turn shapes the active site) Therefore, at lower pHs of the experiment, the rate of reaction was not as quick as the pH was far from the optimum pH (what the pH works at best).

The enzyme was slightly denatured as secondary bonds holding the protein together were disrupted by the free H+ ions which were capable of forming equally strong or stronger bonds with the groups holding the trypsin together. This process was shown as in the pH of 4. 5; the rate of reaction was 2. 9 whereas at the optimum (pH 8. 8) the rate of reaction was 58. 8 (nearly 20 times faster). This helped conclude that trypsin is found in the duodenum where it works at its optimum condition.

The pH is very alkaline in the duodenum as it has to neutralise the acidic hydrochloric acid that is made in the stomach. Hydrochloric acid is produced in the stomach to destroy harmful bacteria and this makes the enzymes in the stomach work best in its acidic conditions (pH 2). The duodenum produces sodium hydrogencarbonate that provides the alkaline conditions for the trypsin to work in. This reaction happened because the casein was the actual substrate for the enzyme trypsin to work on. This means that if another substrate was used which was not a protein, the enzyme would not catalyse the reaction.

This is because enzymes are very specific and they can even distinguish mirror-image (enantiomers) of same molecule so they will not break down the “ fake” substrate. Enzymes are thought to reduce the activation energy in a reaction. They work by “ grabbing” the substrate molecules and attracting them to their active site by electrostatic attraction as they are polar meaning they have a charge that attracts the opposite charge. This was how the casein reacted with the trypsin. There are two theories that show how enzymes work and are shown below. Hypothesis on how enzymes work In 1890 Emil Fischer postulated the lock and key analogy.

He said that the enzyme was to be the “ lock” (Trypsin) and the substrate to be the “ key” (Casein). As mentioned before, the enzymes are specific so only the right shaped “ key” (Casein) could fit into. The site of the lock is called the active site. The theory also states that a reaction cannot happen if the enzyme-substrate complex does not form. However in 1959 Koshland suggested an Induced Fit hypothesis. This hypothesis stated that the substrates were vaguely the same shape of the active site but when the enzyme is enters the active site, it moulds the shape of the active site to allow more efficient binding and catalysis.

These theories helped explain what went on when the casein binded with the trypsin and this helps show that the casein was the right shape for the trypsin in my experiment. So what actually happened in the experiment was the casein fitted into the active site by any of the mentioned hypotheses and the enzyme hydrolysed the peptide bonds that held the casein together

breaking it into amino acids making the solution go clearer therefore letting more light through.

Evaluation The experiment can be judged a success as a general trend was discovered. However, an anomaly was found as it can be seen in figure 5. This was discovered on the pH of 5. 9. However it was not an anomaly for an individual repeat but the average as all the results for pH 5. 9 were nearly the same showing that the technique was not wrong three times. It can be deduced as an anomaly as a straight line was expected but this spoils the trend and the pattern.

The anomaly could have been obtained due to many reasons. One reason is the contamination of the buffer solution. The temperature would have fluctuated as no precautions to ensure the temperature was constant were take and so a decrease in temperature would explain the slightly lesser rate of reaction of the anomaly as colder environments mean that the particles in the solution do not have as much kinetic energy so they do not move around as fast reducing the chance of enzyme-substrate complexes being formed.

The fluctuation in temperature could have given this anomaly in theory but it would have had to be a significant change. The repeats done for the investigation were sufficient as a general trend was discovered but if the experiment were to be done again, more repeats would be done and their averages found out to get even more reliable results and to ensure that even the slightest anomalies did not affect the results. However it is not known if pH of 8. was actually the optimum pH therefore higher pHs should be

considered for future experiments. As mentioned before the temperature was not monitored so in future, a water bath would be used to make sure the temperature was constant to ensure it did not interfere with the rate of reaction. Higher temperatures mean that more kinetic energy is given to the particles making them move around more and quicker making more successive collisions are possible thus increasing the rate of reaction.

The colorimeter was very accurate and sensitive and was affected by light from the environment around so the experiment was done on the same day to ensure that the climate and conditions were the same. However, it is a possibility that the slight changes in light would have changed the amount of light transmitted through therefore increasing the chance of human error. Another aspect that could be considered is the use of a flea (magnetic stirrer) that would mix the solution more uniformly for each experiment ensuring more reliable results.

In the experiment the testube was shaken for each repeat as evenly as possible but it was not as accurate as using a magnetic stirrer and therefore due to human error the shaking would have affected our results showing a change in the rate of reaction. (More mixing means more chance of an enzyme substrate collision). In future attempts a different range of pHs could be used (for example: 4. 1, 4. 2, 4. 3, 4. 4... instead of 5. 9 then 7. 0) so that it would help confirm a stronger trend as there would be more points to plot on the graph.

Going beyond the pH of 8. would show if the optimum was really pH 8. 8 or if the rate of reaction did increase on other pHs beyond 8. 8 and when the enzyme actually becomes denatured. Also investigating how different factors affect the rate of reaction with the enzymes like the change of temperature or the concentration (instead of 2% protease) would be considered to illustrate the characteristics of enzymes. However all the aspects mentioned above could have affected the results but a general trend was discovered therefore showing that they really did not have a huge affect.