

Enzyme kinetics laboratory report



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How the changes in substrate concentration (PNPP), changes in temperature, changes in pH and the presence of an inhibitor (phosphate ions) effects the rate of reaction of the reaction between PNPP and water catalysed by the enzyme acid phosphates which produces PNP and phosphate as its products . Using spectrophotometer to measure the absorbance of the products formed through hydrolysis of substrate.

Abstract:

The purpose of this scientific paper was to replicate earlier findings of experiments in enzyme kinetics and to see if enzyme behaviour and activity is influenced by 4 factors- change in substrate concentration, temperature, pH and the presence of a competitive inhibitor. In the first section where the substrate concentration was altered whilst the enzyme concentration was kept constant the relationship was found to be hyperbolic – this can be explained using the Michaelis -Menten equation and the Lineweaver burk plot which was also used to obtain K_m values of 0. 25 and V_{max} values of 0. 12UNIT. The increase in $[S]$ increased the rate of reaction at first but when $[S]$ too much the initial velocity started to decrease. The increase in temperature led to a general increase in the initial velocity until the temperature reached 347 K where the enzyme denatured. The optimum temperature of the reaction was in the range 310 K- 323. 2 where the rate increased at its fastest. The third section was the effect of pH on the enzyme activity. The optimum pH of the reaction was 5. 5. This pH gave the highest reading of absorbance which means that the rate of hydrolysis was at its highest. Finally the last section of the experiment was the effect of competitive inhibitor on the rate of reaction. The investigation showed that the inhibitor used (phosphate ions) was a non-competitive inhibitor. The <https://assignbuster.com/enzyme-kinetics-laboratory-report/>

inhibitor did lower the initial velocity of the reaction when it attached to the enzyme at random.

Introduction:

This laboratory report is on the topic of enzyme kinetics, previous work in this particular field was carried out in the 1700's, when biological catalyst enzymes were discovered and studied. As understanding of enzymes increased scientist carried out basic laboratory experiments such as the ' conversion of starch to sugar by saliva' to increase their understating of enzymes and how they function. However the first actual incident where enzymes were understood in detail was in 1897-by Edward Buchner . Since then on, till this period of time factors which effect the velocity/ rate of reaction and enzymes have been studied widely by many scientists globally. The modern scientists have a deep understanding of enzyme kinetics. The purpose of this laboratory report is to confirm previous findings covered in various sources of scientific literature and journals.

Enzymes are often described as ' organic catalysts which increase the rate of reaction of a bio-chemical reaction' (David L. Nelson etal 2008). Enzymes increase the rate velocity of various reactions that occur in a biological system such as the mammalian digestive system. Enzymes can have functions including transfer, synthesis or breakdown of molecules. It is important to mention that enzymes are proteins which speed up the rate of reaction without being used up themselves i. e. they are reusable. Enzymes have a very specific active site which is complementary to a specific substrate 3 dimensional structure.

The specificity is due to the complementary hydrophilic/hydrophobic charge, electrical charge and shape of active site on the enzyme. The binding of an enzyme with a specific substrate produces an enzyme substrate complex (ES). The rate at which the enzyme substrate complex is formed is dramatically increased or decreased in changes in substrate concentration, temperature, pH and presence of a competitive inhibitor. The effect of these 4 factors on the enzyme activity is known as enzyme kinetics (Jeremy M. Berg et al 2006). Enzymes work on the concept of transition states. Transition state of enzymes is the phase where the substrate is not yet a product and not a substrate. Enzymes reduce this phase. The difference between the free energy of the reactants and the free energy of the transition state is the activation energy (E_a). The minimum energy required for a successful reaction to occur. Enzymes speed up the rate of reaction by lowering the activation energy barrier.

A typical enzyme substrate reaction can be simplified and be written as:

The enzyme studied in this investigation was acid phosphatase. This enzyme can be identified by its 'Enzyme Commission Number' (EC number) which is 3. 1. 3. 2. Now I will briefly discuss the enzyme kinetic properties of acid phosphatase. The enzyme acid phosphatase catalyses the removal of phosphate group from organic molecules. Its primary mechanism of action is to bring a substrate molecule into contact with a molecule of water. This results in catalysis and the removal of phosphate group. Finally a hydroxyl group is attached to the substrate molecule. An example of this is the reaction shown below. This reaction was studied in this laboratory investigation.

Acid phosphatase

p-nitrophenylphosphate (PNPP) + water phosphate + p-nitrophenol(PNP)

In the reaction above PNPP is the synthetic substrate, which is described as chromogenic. At high purity this compound is completely colourless. PNPP is hydrolysed by the enzyme acid phosphatase this produces phosphate and PNP. The product produced is yellow and can be measured using a colorimeter.

The first part of the investigation concerns the effect of substrate concentration on the rate of reaction. I predict and expect that at an increase in substrate concentration (PNPP) will lead to an increased rate of reaction/initial rate. The reason for this is that an increase in substrate concentration increases the chances of a successful collision occurring between the enzyme and substrate increasing the likelihood of ES complex and product (PNP) formation results in an overall increase rate of reaction. This will produce a first order reaction on a graph. However it is important to mention that at high substrate concentration the enzyme will be fully saturated this will reduce the rate of reaction as there are too many substrate than enzymes. On a graph this would show a plateau. The maximum velocity of the reaction or the V_{max} would be expected to be close to this region. Therefore I predict to see a hyperbolic relationship on a graph.

Section B of this investigation concerns the effect of temperature on the rate of reaction. I would expect to find that an increase in temperature would result in an increase in the rate of reaction. The reason for this phenomenon

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is that as there is increase in kinetic energy being applied to enzymes and substrates it increases the chances of collision occurring so more product (PNP) is formed per unit time. However I also believe that temperatures above 50 -70 degrees Celsius would denature the enzymes active site and the 3 dimensional structures. At this stage the enzyme will not be complementary in shape to the substrate.

This would mean that no reaction can be completed so the rate of reaction will decrease. It is important to state that the enzyme is going to have an optimum temperature at which the ES complexes and products are made at the fastest velocity. The increase in temperature increase the amount of molecules which have higher energy than the E_a barrier this in turn increases the amount of molecules which can react increasing the rate of reaction or initial velocity. I believe the optimum temperature is going to range between 20-40 degrees Celsius.

The third part of this lab report is based on the effect of pH on the rate of reaction again I believe that at extremes of pH such as acidity or alkalinity will affect the rate of reaction. This may cause the enzymes structure to denature and will cause a lower rate of reaction and lower rate of product formation because the enzyme will not be complementary to the substrate. I would expect this enzyme to have a low optimum pH of around 5-2 as it is acid.

Finally the last section of this investigation concerns the effect of a competitive inhibitor (phosphate ions) on the rate of reaction. Inhibitor in this case the phosphate ions will compete with the actual substrate PNPP for the

active site of the enzyme acid phosphatase. These inhibitors can only attach to the active site of the enzyme due to their complementary properties. The concentration of the inhibitors will be kept constant and the concentration of the substrate PNPP will be altered. I believe that as the concentration of PNPP increases the rate of reaction will increase this is because the PNPP can out- compete the competitive inhibitor and reverse the effect of the inhibitor.

Materials and Methods:

All procedures were carried out as described in the lab schedule. No alterations were made to any of the procedures carried out throughout the experiment.

Discussion:

The first section of this lab report relates the effect of substrate concentration on enzyme activity. I shall briefly discuss and explain what my data shows and means. Graph 3 which is the graph of Michaelis Menten of collected data. The relationship between the rate of an enzyme-catalysed reaction and the substrate concentration can be describes as hyperbolic. The graph and tables 2 show that as the concentration of substrate (PNPP) increases from 0. 00 Mmols to 0. 20 Mmols of substrate the initial velocity or the rate of reaction shows an increase in too. The relationship can be describes as fist order as the $[V]$ rises almost linearly with the increase in $[S]$.

However after the concentration increase from 0. 20- to 3. 00 Mmols the initial velocity shows a gradual increase and then gradually levels of or slopes here graph shows 0 order relationship. This supports the prediction I made in the introduction. The graphs indicates that as the concentration of substrate increase the rate of reaction increase this is because of the

increase chances of a collision between a substrate and an enzyme. This in turn increases the rate at which ES and P are formed. However after the concentration increase after 0.20 3.00 Mmols the rate starts to level off. The reasoning behind is this is that there are too many substrates than compared to enzymes and all enzymes are described to be fully saturated. At this phase the reaction is at its Maximum velocity and cannot increase in rate unless more enzymes are added therefore enzyme concentration is the limiting factor.

I can tell that the data I collected from the experiment in the lab is accurate and valid as the graph which compares the theoretical data and the collected data show similarities in the trend line the V_{max} the K_m and as well as the points and there is no anomalous data point on either of the 3 graphs. My findings support the findings of many previous investigations and journals as the graphs show a similar relationship as to the findings to previous work. The implications of this investigation can be used widely to determine the V_{max} and K_M . These values can be used for medical purposes since the enzyme acid phosphatase is manufactured by the body to remove phosphate groups.

The limitation of Michaelis Menten plot is that the K_m and V_{max} cannot be accurately determined. Therefore a Lineweaver burk plot is plotted. The Lineweaver burk plot shows that the K_M and V_{max} are very similar to the ones from the Michaelis Menten plot.

Finally K_M is an approximate measure of the affinity of an enzyme for its substrate. A small value of K_M indicates a high affinity of the enzyme for the

substrate. From my graphs (1 +2) I can tell that the K_M was 0.24 and therefore small. This means that the enzyme acid phosphatase has a high affinity for the synthetic substrate PNPP. This means that small concentration of substrate PNPP is enough to run the reaction at half of maximum velocity ($1/2 V_{max}$).

The next section of the investigation concerns the effect of temperature on the rate of reaction. I predicted an increase in temperature will increase the rate of reaction or the initial velocity of the reaction until the enzyme denatures and the protein sequence becomes damaged and unravels. The actual reason for why the protein sequences unravel is that the protein has too much kinetic energy and the amino acids vibrate and this causes the non covalent forces to be weakened. There is an increase in the rate of reaction as the temperature increases from 278 -323 K the increase is almost linear. However after the temperature 323 K-to 347 K the enzyme denatures and unravels. This is proved by the last point on the graph which doesn't fit the line of best fit. Overall the graphs and data all prove my predictions correct. There is no other anomalous data present.

The third section concerns the effect of pH on the rate of reaction. As we have discovered before the enzyme used was acid phosphatase so the pH at which it works at best - the optimum temperature will be in the acidic pH regions. I can confirm from my graph that the optimum pH of the reaction is in the region 5-5.5 which is indeed acidic. pH of 5.5 gives the highest absorption value, this therefore means that the rate of hydrolysis was at its fastest.

A possible explanation for the change in the experimental design is that the pH may denature the enzyme if the enzyme was added first the active site and 3 dimensional shape of the enzyme could be altered at extremes of pH such as 2 which is quite acidic. The pH does not affect the substrates 3 dimensional structure in the same ways as the enzymes. Therefore to obtain valid results /data the design for this part of the experiment was slightly altered.

pH has a very strong effect on enzyme activity . This is because enzymes work on the basis of mechanisms such as induced fit hypothesis and lock and key . The enzymes have a specific active site due to the complementary 3 dimensional active site with the substrate. According to the induced fit hypothesis the enzyme can undergo some change in the 3- dimensional structure in order to fit the substrate. However a change in pH changes the enzymes active site structure. An increase in H^+ ions can change the active site structure due to the fact that H^+ ions can interfere with any polar molecules on enzymes protein structure. Amino acids side chains can be protonated and may be damaged due to the extreme pH (concentration of H^+)this may result in the protein sequence to unravel which means that the active site is not complementary to the substrate and the rate of reaction decreases as no or little ES are formed which results in little products formed.

The final section concerns the effect of a competitive inhibitor (phosphate ions) on the rate of reaction. From my graph I can tell that the inhibitor present was an non-competitive inhibitor. I can tell this by looking at the Lineweaver Burk plot where the trend lines cross the $1/V_{max}$ intercept at <https://assignbuster.com/enzyme-kinetics-laboratory-report/>

different values but cross the $1/k_m$ intercept at the same values. This means that this type of inhibitor does not affect the K_M but does lower the V_{max} values.

Non competitive inhibitor has the same effect as lowering the total volume of enzyme. This type of inhibition is reversible. It binds to a site away from the enzymes active site it denatures the active site so no other substrates can bind to the active site. This in turn lowers the rate of reaction and the rate at which products are formed. However this type of inhibitor also binds to the enzyme substrate complex I can therefore conclude that the non competitive inhibitor does lower the rate at which the products are formed.

References:

Books:

1. David L. Nelson et al (2008) Lehninger Principles of Biochemistry , W. H Freeman and Company
2. Jeremy M. Berg et al (2006) Biochemistry, W. H Freeman and Company
3. Lab schedule (2009) – Enzyme Kinetics MODULE LSC10034 –
EXPERIMENT 4

Lecture Notes:

1. Lectures notes (2009) Dr John Mills- Module LSC-10034 Proteins and Enzymes – lectures 9, 10, 11 (Figures 1-5)
2. Absorbance values- Table 4 and Table 6 – Emma Ezekiel

Web Pages:

1. EC Number -[http://www.brenda-enzymes.org/php/result_flat.php4?ecno= 3. 1. 3. 2](http://www.brenda-enzymes.org/php/result_flat.php4?ecno=3.1.3.2) – Seen : 20 November 2009