

Analyzing The Activity Of Pyrophosphates Enzyme

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Enzymes are biological globular proteins that catalyze the rate of biological reactions. Enzyme kinetics analysis the rates and factors affecting the rate of enzyme activities on the enzymes on the substrates. This can be investigated under varying conditions under which the reaction takes place. Factors affecting the rates of the enzymatic reactions include substrates and enzyme concentrations, temperature, pH, and inhibitors. The mechanism of the enzyme activity is that the enzyme binds with the substrate at the active site, forming an enzyme-substrate complex, which later dissociates to form the product and the free enzyme. An enzyme is never used up or changes the equilibrium level but only alters with the time taken to reach the equilibrium. The aim of this experiment is to study the enzyme kinetics of the enzyme pyrophosphates with acid molybdate. The experiment analyzed the time taken to complete the reaction under different reaction conditions. These conditions, which include temperature, pH and the substrate and enzyme concentration, affect the binding of the substrate to the enzyme and the turnover rate. Inorganic pyrophosphates, named EC 3.6.1.1, catalysis the hydrolysis of pyrophosphate (PPI) that proceeds as a bioproduct in many biochemical syntheses utilizing ATP as the source of energy. The enzyme splits a pyrophosphate molecule to form two phosphate ions in the presence of divalent metal cations and in a highly exergonic reaction .pyrophosphatase acts as a phosphoryl group donor. (Laidler & Bunting, 1973) The enzyme pyrophosphatase has critical roles in the living organism. (Tóth et al., 2007; VOLK et al., 1982)

The enzyme plays an essential role in lipid metabolism, DNA metabolism, the formation of bones and neuron growth. Due to its exergonic nature, it is associated with various types of cancers and tumors, such as ovarian, lung and brain cancer. Pyrophosphate act as phosphoryl carrier DNA replication, pyrophosphate is a byproduct of DNA formation to form new DNAs and pyrophosphate(PPI).PPI removes deoxyribonucleosides from DNA to form dNTPs.in fatty acid metabolism, a molecule ATP reacts with fatty acids to form acyl adenylate. Acidified ammonium molybdate is used to quantitatively determine the presence of pyrophosphate in a color reaction. Pyrophosphate is highly reduced by blue molybdous acid and compounds.

Material And Method

In preparation for the standard curve, different phosphate concentration was used to react with a constant 2.5 ml of molybdate acid for ten minutes for the blue color to form. The intensity of the color was measured at 620 nm. The blank solution was prepared the same way as the reagent solution but lacked phosphate.

In investigating the effect of enzyme concentration, different amount of pyrophosphate enzyme was

added. Magnesium chloride and triethanolamine buffer were used. On the effect of pH, the experiment was carried at varying pH condition. Other factor was kept constant but the buffer solution not included. In investigating the temperature activity, the experiment was carried out at different temperature conditions, but previous factors were held constant.

Result Presentation

On the Effect of enzyme concentration, after carrying out the experiment and measuring the absorbance, an increasing absorbance with the increasing enzyme concentration over the successful texture was noted. Absorbance levels increased with increasing concentration in both sets of experiments. However, data varied slightly between the two sets apart from the 4th test tube. After calculating the rate of reaction with the substrate and enzyme concentration, the rate increased over the successful from test tube 1 to test tube 5. The graph drawn for the average hydrolysis against the enzyme concentration formed a straight line with linear progression. The trend line was developed after joining the line of best fit and had a positive gradient starting from the origin. There were also anomaly spots deviating slightly from the line.

On the pH. The effect of absorbance increased with the increasing acidic pH until an optimum was reached at neutral pH. Further, increase with the basic pH. Reduced the absorbance steadily. The rate of hydrolysis also increased with the increasing pH. Up to the optimum neutral point. After which, further, increase in the pH. Caused a decline in the rate of reaction. After graphing of the rate of hydrolysis against pH. was drawn, it presented a curve with the optimum rate at the 7sevenpH mark. There was no trend line developed.

The effect of pH showed similar results. Absorbance increased with the increase in temperature until an optimum point of 70 degrees was reached. An increase of temperatures to 100 degrees reduced the absorbance. Absorbance deviated slightly between the two sets of data. The rate of hydrolysis increased with the increasing temperatures up to the optimum point but reduced gradually with the increase in temperature. However, the rate at 100 degrees was higher than at 4 degrees Celsius, with both enzymes present at both conditions. The graph drawn with the rate of hydrolysis against pH was optimum at 70 degrees. No trend line developed.

Results



Table 1: Effect on enzyme concentration



Table 2: Effect on pH



Table 3: Effect on temperature



Figure 1



Figure 2: graph on the effect of ph. on enzyme activity



Figure 3: Effect of temperature on the effect of enzyme activity

Discussion

Enzyme activity is affected by the various factors of enzyme concentration, pH and temperature. The amount of enzyme concentration present affects the rate of a catalytic reaction with an increase of the reaction with the increasing substrate concentration and a lowering of the rate with a decrease of enzyme concentration. The enzyme catalyzes the reaction by binding to the substrate's active site. Increasing the enzyme concentration increasing the chance s of enzyme combining with the substrate to form the product and the free enzymes. However, an increase in the enzyme concentration suppressing the substrate concentration can cause a further increase, making the enzyme concentration the limiting factor in the reaction.

Temperature affects the rate of enzyme catalyzing the reaction by interfering with the enzyme. [Temperature interferes](#) with the stability of the bonds holding the enzymes together. Temperature also affects the kinetic energy of the reaction molecules, hence affecting the rate of collision and reaction. The different enzyme works best at certain optimum temperature conditions. An increase in temperature increases the rate of enzymatic reaction until an optimum point, after which the rate lowers. The decrease in temperature also reduces the rate of enzymatic reaction. Since the enzyme is a protein in nature, an increase in temperatures beyond the optimum points completely destroys the arrangement and organization of the enzyme structure, hence denaturing it. When temperatures also go below the optimum, it affects the structure of the enzyme, rendering it inactive. However, an increase in temperature will activate the enzyme to normal working.

Ph is also a factor affecting the rate of enzymatic reaction. An increase in pH increases the rate of enzymatic reaction until an optimum point is reached, after which a further increase in the pH ionic strength slower the rate of chemical reaction. Each group of enzymes has its optimum pH working condition. Some enzyme works optimally in basic conditions, others in neutral pH, and others in acidic conditions. Ph may affect both the structure and shape of the enzyme and substrate, causing the enzyme not to fit in the active site of the enzyme. This affects the rate of the reaction.

Data from the experiment correspond to the above theory lecture. According to Figure 1, enzyme concentration increased with the increase in enzyme concentration. In Figure 2 increase in p increased the enzymatic reaction until an optimum pH of 7. According to Figure 3, an increase in temperature increased the rate of chemical reaction until the optimum of 70 degrees. After this enzyme becomes a denatured and further increase in temperature, declining the rate of reaction. However, there were some anomalies in the experimental data.

In collecting the anomalies, a more precise method of measurement can be used. Molybdenum compounds highly reduce pyrophosphate compared to the acid itself. The experiment can also be harnessed by the use of modulators, and the effect on substrate concentration and the effect of inhibitors can also be studied.

Conclusion

The experiment was a success in analyzing the effect of pyrophosphatase enzyme with acid molybdenum. The aim of the experiment was to analyze the effect of pyrophosphatase enzymes, and the aim was achieved. Though slight human errors cannot be avoided, the observation of the experiments corresponded well with the expectations.

Appendix

A standard phosphate solution of 1mM /MI had been prepared, and diluting this solution with different volumes of dilute water resulted in a different concentration of the phosphate solution. Different concentrations can be calculated with the formula.

$$C_1V_1=C_2V_2$$

Phosphate volume	Phosphate concentration
0	0
0.20	0.2
0.40	0.4
0.60	0.6
0.80	0.8

1			1	
Abs 1	Abs 2	Average Abs	Range	
0.000	0.000	0.000	0.000	
0.036	0.048	0.042	0.006	
0.114	0.104	0.109	0.005	
0.158	0.163	0.161	0.003	
0.209	0.207	0.208	0.001	
0.259	0.256	0.258	0.002	

The absorbance measured showed different results for the different test tube reactions. The absorbance increased down the tubes for the standard reactions. Data deviated slightly but in a range of (0-0.002). data was presented in graph and line of best fit formed a straight line, the line developed had a linear equation which can be presented in form of $Y=MX+C$. The linear equation developed can be presented as shown below.

$Y=0.2602x$, where y is the x-intercept while x is the gradient.



Figure a: standard curve

Reference

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