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Biochemistry Laboratory Report

Experiment 8: Analysis of Kinetics of *β-Fructofuranosidase* Based on pH, Inhibitors, Concentration of Enzyme and Concentration of Substrate

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The mechanism of enzyme catalyzed reactions is often studied by making kinetic measurements on enzyme-substrate reaction systems. These studies include measuring rates of the enzyme-catalyzed reactions at different substrate and enzyme concentrations. Studying the kinetics of enzyme is important to know, both to understand enzyme activity on the macroscale and to understand the effects of different types of enzyme inhibitors. In this experiment we tried to analyzing the effects of different pH of the solution, inhibitors, concentration of the enzyme and the concentration of substrate on the kinetics of β-Fructofuranosidase enzyme.

1. INTRODUCTION

Enzymes are very efficient catalysts for biochemical reactions. They speed up reactions by providing an alternative reaction pathway of lower activation energy. Like all catalysts, enzymes take part in the reaction - that is how they provide an alternative reaction pathway. But they do not undergo permanent changes and so remain unchanged at the end of the reaction. They can only alter the rate of reaction, not the position of the equilibrium. Most chemical catalysts catalyse a wide range of reactions. They are not usually very selective. In contrast enzymes are usually highly selective, catalysing specific reactions only. This specificity is due to the shapes of the enzyme molecules. For two molecules to react they must collide with one another. They must collide in the right direction (orientation) and with sufficient energy. Sufficient energy means that between them they have enough energy to overcome the energy barrier to reaction. This is called the activation energy. ppt logo Reaction profile Enzymes have an active site. This is part of the molecule that has just the right shape and functional groups to bind to one of the reacting molecules. The reacting molecule that binds to the enzyme is called the substrate. An enzyme-catalysed reaction takes a different 'route'. The enzyme and substrate form a reaction intermediate. Its formation has a lower activation energy than the reaction between reactants without a catalyst. Enzyme kinetics is the study of reaction rates (time-dependent phenomena), these rates of reactions are affected by concentration of substrates, effectors (activator & inhibitor), temperature and concentrations of the enzyme itself. Studying enzyme kinetics is very useful to understand the quantitative description of biocatalysis, underatnd the catalytic mechanism, understand the regulation of activity and to find the effective inhibitors.

2. EXPERIMENTAL PROCEDURE

Standard Preparation and Standard Absorbance Measurement

6 different tubes were prepared, all labeled with number from 0 to 5, tube 0 is a blank solution and the other tubes contain different amount of mixture of standard (1 mol glucose + 1 mol fructose) and water. Their final volume should be 5ml by fulfilling it with water (0.5, 1.0, 1.5, 2.0, 2.5 respectively for the standard). 2mL of DNS was added to each tube, mixed and heated for several minutes until the color changes can be distinguished. Absorbance of tubes then were determined using spectrometry at 540nm wavelength.

Finding the Optimum pH for the Enzyme-catalyzed Reaction

12 mL of substrate solution (0.1 M sucrose in water) was placed into a seperate test tube and then this tube was placed along with the five from above in a water bath (37^{o}C). These tubes were allowed to reach the temperature of the water bath. The reaction was started by adding 1 mL of substrate to each tube and 10 minutes later 2 mL

of DNS reagent was added to stop the reaction. It is heated for several minutes and the absorbance was measured at 540nm.

Finding the Effect of Concentration of Enzyme on Velocity of the Reaction

10 mL of substrate solution (0.1 M sucrose in buffer) was put into a separate test tube and then this tube was along with the six from above in a water bath (37°C). These tubes were allowed to reach the temperature of the water bath. When this occurs, you are ready to start the reaction. Substrate was used to start the reaction and DNS was used to stop the reaction. After DNS reagent was added the tubes were heated then the absorbance values were measured at 540nm.

Finding the Effect of Inhibitors on Velocity of the Reaction

About 12 mL of β-fructofuranosidase solution was put into a separate test tube and then it was placed along with the 11 tubes from above in a water bath (37°C). These tubes were allowed to reach the temperature of the water. Reaction was started by adding 1 mL of the enzyme solution and 10 minutes later was stopped by adding 2 mL of DNS reagent. The tubes were heated and the absorbance values were measured at 540.

A

Tube no.	Standard (ml)	dH_2O (ml)	Conc (mM)	Abs Value
0	0	5.0	0	0.005
1	0.5	4.5	0.1	0.030
2	1.0	4.0	0.2	0.153
3	1.5	3.5	0.3	0.312
4	2.0	3.0	0.4	0.461
5	2.5	2.5	0.5	0.618

B

No.	Buffered Enzyme (ml)	Buffer Solution (ml)	Abs Value	Conc. (mM)	V (mM/min)
1	0.5	3.5	0.411	0.38	0.08
2	1.0	3.0	0.311	0.31	0.06
3	1.5	2.5	0.981	0.78	0.16
4	2.0	2.0	1.493	1.13	0.23
5	2.5	1.5	1.228	0.95	0.19

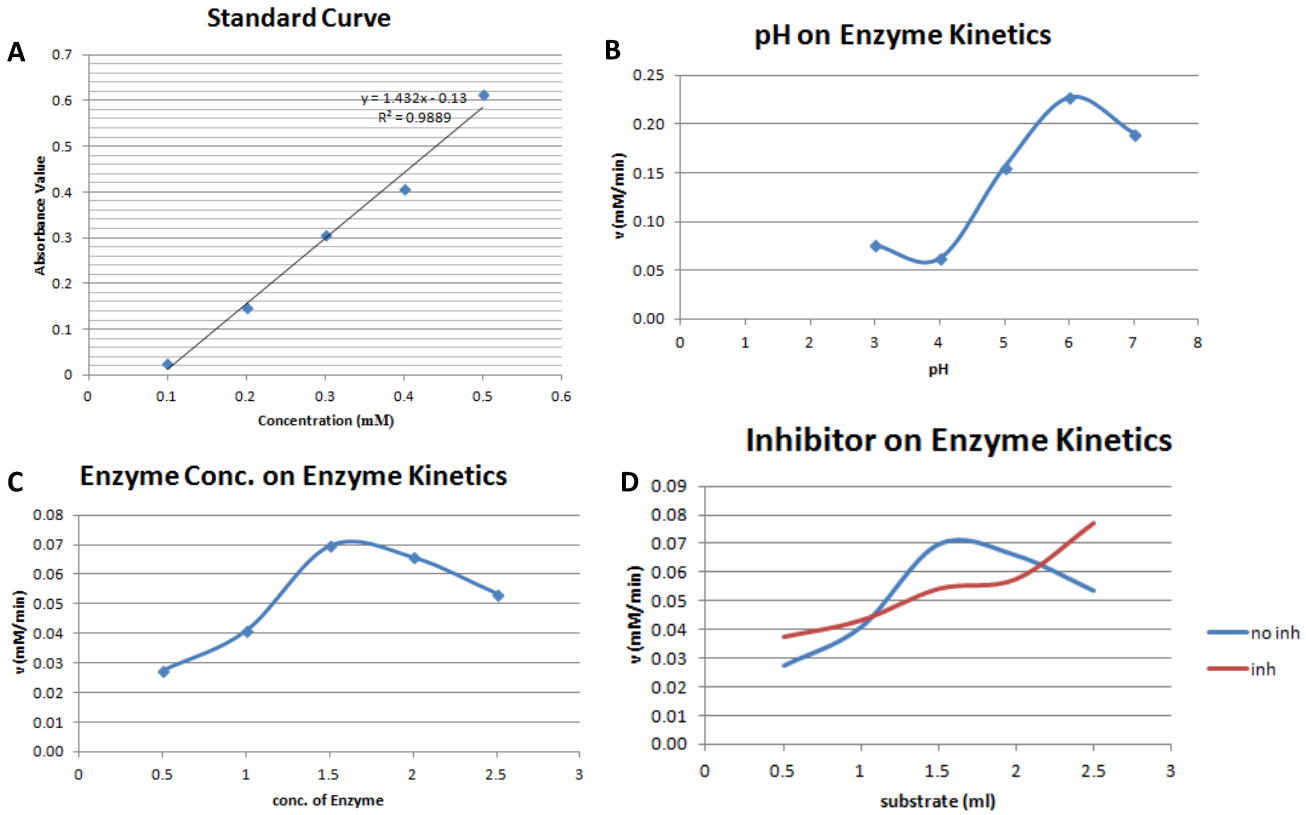
C

No.	Unbuffered Enzyme (ml)	dH2O (ml)	Buffer		Abs. Value	Conc. (mM)	V (mM/min)
			Vol (ml)	pH			
1	1.0	0	3.0	3	0.101	0.16	0.03
2	1.0	0	3.0	4	0.270	0.27	0.06
3	1.0	0	3.0	5	0.135	0.18	0.04
4	1.0	0	3.0	6	0.042	0.12	0.02
5	1.0	0	3.0	7	0.406	0.37	0.07

D

No.	Buffered 0.1M Sucrose Solution (ml)	5M $AgNO_3$ Solution (ml)	dH2O (ml)	0.1M CH_3COOH pH 4.7 (ml)	Abs Value	Conc. (mM)	V (mM/min)
1	0.5	0	1.0	2.5	**0.066**	0.14	0.03
2	1.0	0	1.0	2.0	**0.163**	0.20	0.04
3	1.5	0	1.0	1.5	**0.369**	0.35	0.07
4	2.0	0	1.0	1.0	**0.341**	0.33	0.07
5	2.5	0	1.0	0.5	**0.253**	0.27	0.05
1i	0.5	1.0	0	2.5	**0.140**	0.19	0.04
2i	1.0	1.0	0	2.0	**0.181**	0.22	0.04
3i	1.5	1.0	0	1.5	**0.259**	0.27	0.05
4i	2.0	1.0	0	1.0	**0.283**	0.29	0.06
5i	2.5	1.0	0	0.5	**0.421**	0.38	0.08

Tables 1 showing the ratio of concentration to enzyme in the tubes.
Table 1A. Table for making standard curve
Table 1B. Table for pH on enzyme kinetics
Table 1C. Table for concentration of enzyme on enzyme kinetics
Table 1D. Table for inhibitor on enzyme kinetics
All tables contain absorbance values, measured at 540nm. Table B to Table D contain concentration which is the interpretation of the standard curve and v (velocity), concentration divided by 5, because the reaction was left to occur for 5 minutes.



Figures 1 depict the interpretation of data from tables 1 in scatter plots. All represent their own data (ex: **Figure 1A** represents **Table 1A**).

3. RESULTS

Concentration of the solution was measured by substituting the absorbance values to the linear equation obtained from the standard curve. The velocity was calculated based on the fact that the reaction was let to occur for 5 minutes, thus to calculate the velocity of each reaction, they only need to be divided by 5. In the pH on enzyme kinetics the velocity of the reaction appeared to be not constant, by not constant it means that sometimes the velocity of the reaction sometimes increased and sometimes decreased. This is contrary to the fact that the curve should have had a bell-like shape, but in this case it has no inconsistent shape instead, the probable reasons that caused this will be explained in the discussion part.

In the enzyme concentration on enzyme kinetics experiment the velocity increased for the first three tubes but decreased after, this is also contrary to the fact that the velocity should have reached max velocity once it cannot catalyze the substrates further and the graph should have been linear after it reached the maximum velocity of the reaction, it is also learned that the concentrated solution the faster the reaction to occur, this is because of the fact that more concentrated solution the solution gets the more enzyme in the solution is. In inhibitor on enzyme kinetics experiment the graph was also not consistent, the ones with inhibitor should have taken a longer reaction time but it didn't.

4. DISCUSSION

We can confirm that the experiments were failed, because the graphs didn't appear as it is supposed to be. This failure can be caused by several reasons; for example, the solution was contaminated with other substance when the experiment was conducted, this is a fatal error because DNS is very sensitive to reducing substance, another reason could be the fact that it took to long for the solutions to be reach the desire color when it is heated. Absorbance measurement error could also happen in the experiment for example like dirty vial tube or pipette errors.

5. REFERENCES

Williams, Lorens. 2014. 'Biochemistry I; Chapter 12: Enzyme Kinetics'.