An Investigation of Catalase/Hydrogen Peroxide Enzymatic Kinetics

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Abstract

Catalase is an enzyme that breaks down hydrogen peroxide into water and gaseous oxygen. The research outlined in this paper investigated this property of catalase and verified whether or not this enzyme/substrate relationship follows the Michaelis-Menten relationship. This was carried out by observing the effects catalase had on graded concentrations of hydrogen peroxide with the use of a proxy. After observing the reactions among differing concentrations of hydrogen peroxide and recording the results, it was found that the data did not fit into the Michaelis-Menten relationship. However, this was most likely due to human error and the data was arbitrarily tweaked to make it fit the relationship.

Introduction

All organisms are limited by the chemical reactions that make up their metabolism. These reactions break down the energy from food consumption or photosynthesis into a usable form. However, these reactions do not spontaneously occur. They are also limited by the amount of energy that must be supplied to drive them. This necessary input of energy is known as the energy of activation. Reactions that are limited by an energy of activation will occur slowly without any external impetus. Enzymes increase the rate of reactions by lowering this energy of activation.

Catalase is an important enzyme that breaks down hydrogen peroxide (H_2O_2) into water and gaseous oxygen, both of which are relatively harmless to cells. Hydrogen peroxide is a byproduct of many metabolic reactions, including the oxidation of fatty acids in the peroxisome of cells. Catalase must be present in peroxisomes to rid the cell of any harmful hydrogen peroxide. In a study done on fibroblast cells, it was found that a lack of catalase in the peroxisome can lead to neurological disorders stemming from peroxisomal diseases (Faruk G. Sheikh, 1998). A deficiency in catalase can also lead to an increased risk of diabetes and a lower life span (Laszlo Goth PhD, 2000). If the intracellular concentrations of hydrogen peroxide are not regulated by catalase the excess H_2O_2 can react with important cell components, degrading organelles necessary for survival or acting as

an intracellular messenger. This intracellular messaging can create cascades of reactions that are not necessary for cell survival.

An enzymatic kinetics study is an investigation of what correlations might be present between the rate of the reaction and other independent factors. Temperature, pH, inhibitors, substrate concentrations, and more can all affect the kinetics of an enzyme. Enzymatic kinetic studies shed light on the biological interactions at the molecular level. Cytrochrome P450 is a protein that reduces nitric oxide to nitrous oxide and, with the use of spectroscopic and kinetic evidence, a specific mechanism for this reduction has been proposed (Yoshitsugu Shiro, 1995). Spectroscopic measurements interpret the interaction between matter and light. A spectrophotometer sends beams of light through a sample and, with proper calibration, measures the light absorbance of the sample. If a reaction changes the absorptivity of a sample, this absorbance can be correlated to the rate of a reaction.

Another tool to measure rates of reaction is the use of a proxy. A proxy is a variable that has a high enough correlation with the variable being studied that it can be used to study the variable in question. A proxy is utilized when measurement of the proxy is more accessible than measurement of the variable being studied. In this investigation of catalase activity a proxy was used to measure the evolution of gaseous oxygen. A disc soaked in catalase and then submerged in a solution of hydrogen peroxide will be pushed towards the surface by the oxygen bubbles that will form underneath it. The rising rate of the disc has a strong correlation to the amount of oxygen produced. A reaction that happens at a quicker rate will produce more oxygen and the disc is expected to rise faster. This approach provides a less direct, but easier way of measuring the rate of the catalase reaction. This approach can be done without the use of expensive machines that may not be accessible. However, this approach also relies heavily on human precision. Because there is no use of precise machinery there is more chance for human error.

As already mentioned, many factors affect the rate at which a chemical reaction proceeds. The focus of this investigation is how the rate of the reaction changes in accordance to the change in substrate concentration. Many enzymes show an increase in metabolic activity as the concentration of the substrate is increased. Enzymes do not have any sort of methodological movement one way or another; they randomly diffuse through the environments they inhabit. If the concentration of a given substrate is low then the probability of the enzyme encountering the substrate is also low. As the concentration of the substrate increases, the probability of the enzyme encountering the substrate also increases because there is less space for the enzyme to inhabit that is devoid of its substrate. However, the enzyme can become saturated with substrate once the substrate's concentration is

at a high enough level. At this point an increase in substrate concentration will not have a considerable effect on the rate of the reaction. The rate of the reaction will only be affected through other means, such as temperature or pH changes. This phenomenon is depicted through the Michaelis-Menten equation. As the concentration of the substrate increases the rate of the reaction asymptotically approaches its maximum velocity (V_{max}). The concentration of the substrate at which the rate of the reaction is one half the V_{max} is known as the Michaelis constant (K_M). The graph of this relationship as a function of substrate concentration is curvilinear and it plateaus as it approaches V_{max}. The variables of the Michaelis-Menten equation can be manipulated to produce an equation as a function of the inverse of the substrate concentration as related to the inverse of the rate of the reaction. Doing this produces a linear graph known as the Lineweaver-Burk plot. The Michaelis-Menten equation and Lineweaver-Burk plot are useful in doing research that relies heavily on rates. With the use of both of these equations the transport of lysine into yeast cells has been found to occur by two different pathways (Grenson, 1966). The purpose of the research done for this paper is to investigate the Michaelis-Menten relationship between catalase and hydrogen peroxide and to produce a Lineweaver-Burk plot of the results.

Materials and Methods

Preparation of the beef catalase extract

The catalase enzyme was extracted from a beef liver homogenate. Two grams of beef liver were blended with 250 mL of cold 0.1 M pH 7 potassium phosphate buffer. The blender was pulsed to prevent mechanical overheating, which could denature the enzyme. This homogenate was then filtered through three-layered cheese close and 25 mL of the filtrate was obtained. The filtrate was kept cold.

Proxy assay of catalase activity

Ten 50 mL beakers were prepared, containing graded dilutions of H_2O_2 from 0.50 % down to 0.01 %. The final working volume of each beaker was 20 mL. The depth of each solution was measured to the nearest millimeter. This data can be seen in Table 1. For each concentration small paper discs were soaked in the enzyme extract for 10 seconds and then placed at the bottom of the beaker and released. Because the reaction between the hydrogen peroxide on the discs and the catalase started as soon as they came in contact, the discs were lowered to the

bottom of the beaker quickly and released smoothly from the forceps to prevent the disc from being bumped. The rising time of the disc was recorded to the nearest second from the moment the forceps released it to when it reached the surface of the solution.

Results

There was a definite correlation between the rate of the reaction and the hydrogen peroxide concentration. The tabular results of the data can be found in Table 1.

Table 1: This table shows measurements from each beaker of differing concentrations of hydrogen peroxide.

Beaker number	% H_2O_2	Disc rise time 1 (s)	Disc rise time 2 (s)	Disc rise time 3 (s)	Distance traveled (cm)
1	0.5	2.0	1.6	1.9	0.20
2	0.3	4.3	2.3	4.6	0.20
3	0.2	4.8	3.4	3.8	0.20
4	0.1	7.0	10.7	8.5	0.20
5	0.07	13.6	16.2	19.0	0.20
6	0.05	19.3	28.0	28.0	0.20
7	0.04	55.3	40.0	45.6	0.20
8	0.03	40.5	43.2	33.0	0.18
9	0.02	58.0	54.0	78.0	0.18
10	0.01	218.0	292.0	284.0	0.20

Beaker number	Disc rate 1 (cm/s)	Disc rate 2 (cm/s)	Disc rate 3 (cm/s)	Mean rate (cm/s)	Standard deviation (cm/s)
1	0.100	0.125	0.105	0.110	0.013
2	0.047	0.087	0.043	0.059	0.024
3	0.042	0.059	0.053	0.051	0.009
4	0.029	0.019	0.024	0.024	0.005
5	0.015	0.012	0.011	0.013	0.002
6	0.010	0.007	0.007	0.008	0.002
7	0.004	0.005	0.004	0.004	0.001
8	0.004	0.004	0.005	0.005	0.001
9	0.003	0.003	0.002	0.003	0.001
10	0.001	0.001	0.001	0.001	0.000

Because the procedure was repeated three times for each concentration with three difference paper discs a more accurate mean rising rate for each disc was able to be calculated. This mean rising rate for each concentration of substrate can be easily seen in Figure 1.

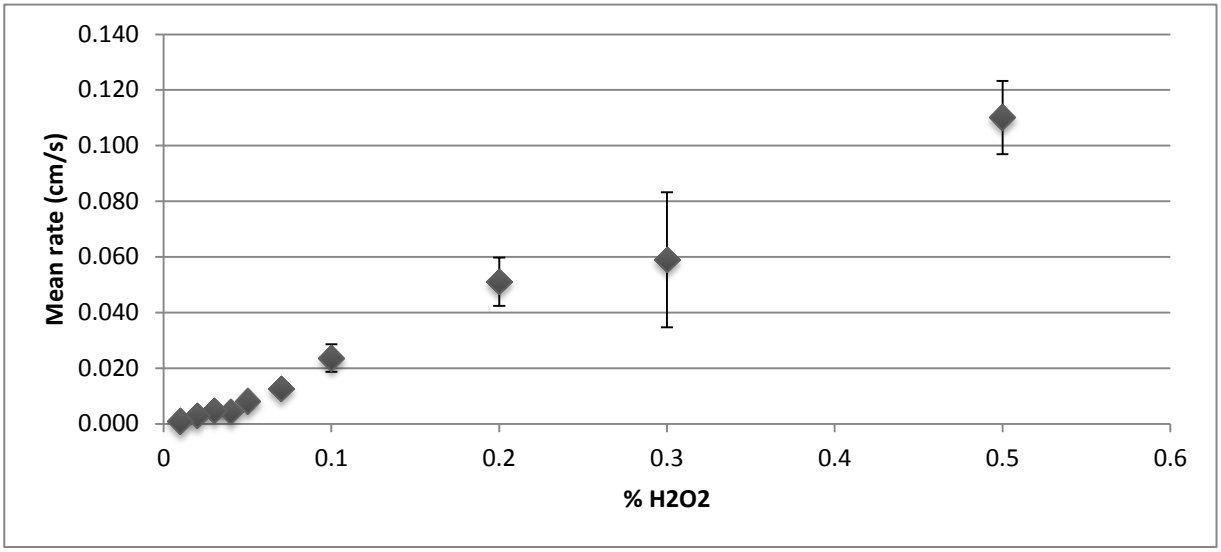

Figure 1: Mean rising rate of discs in different concentrations of H_2O_2

From the recorded the Lineweaver-Burk plot was formatted. The inverse of the concentration of the substrate was plotted against the inverse of the mean rising rate for each concentration of the hydrogen peroxide. This plot, along with the equation of the linear regression line and the correlation index of r^2, can be seen in Figure 2 on the next page. From this equation the V_{max} was found to be -0.011 and the K_M was found to be -0.134.

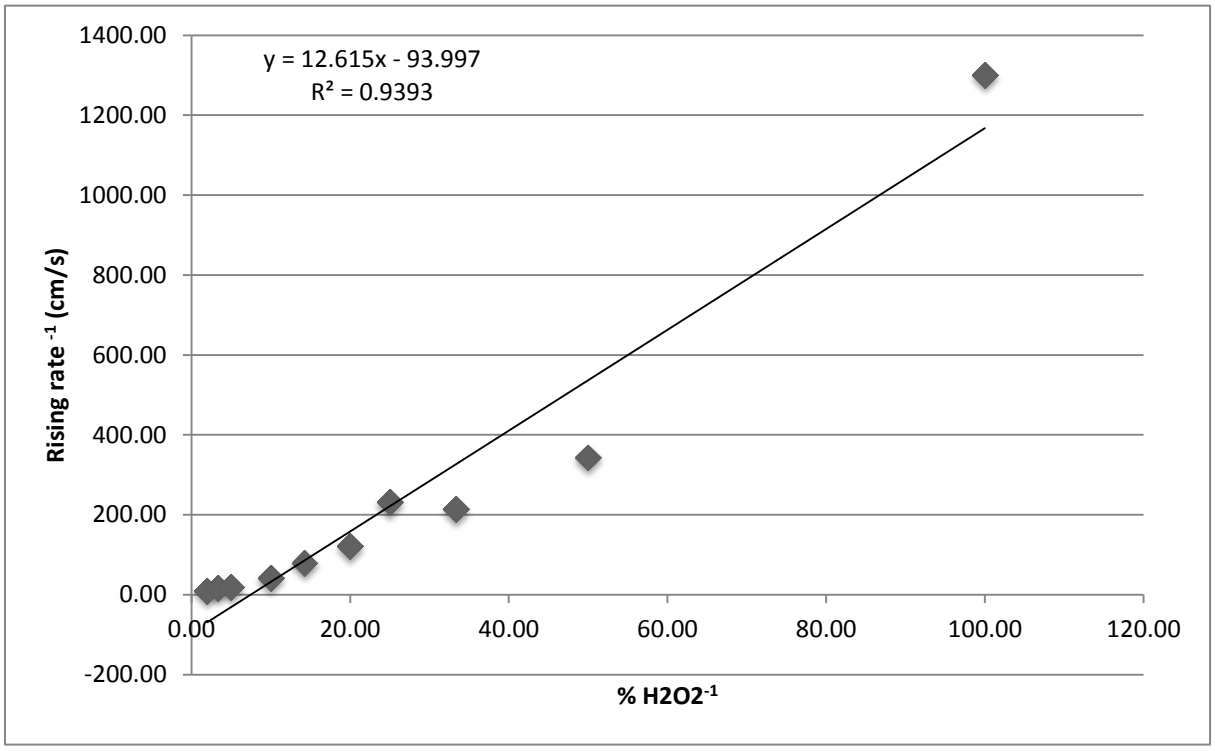

Figure 2: Lineweaver-Burk plot.

When the V_{max} solved to be a negative number a few outliers in the lower concentrations were deleted. The data points in lower concentrations were more likely to be less accurate so deleting them was expected to make the V_{max} positive. Unfortunately, the V_{max} persisted in being negative after multiple data sets were deleted. Because this did not solve the problem the previously deleted data points were reinstated into the graph and the V_{max} was arbitrarily made positive for future calculations. The K_M also solved to be a negative number and throughout the troubleshooting to make the V_{max} positive it never changed to positive either. So the K_M was also arbitrarily made positive for future calculations. These newly calculated V_{max} and K_M were then plugged into the Michaelis-Menten equation and, using Microsoft Excel, expanded on to predict what this relationship would look like up to a substrate concentration of 100% (Figure 3 on the next page).

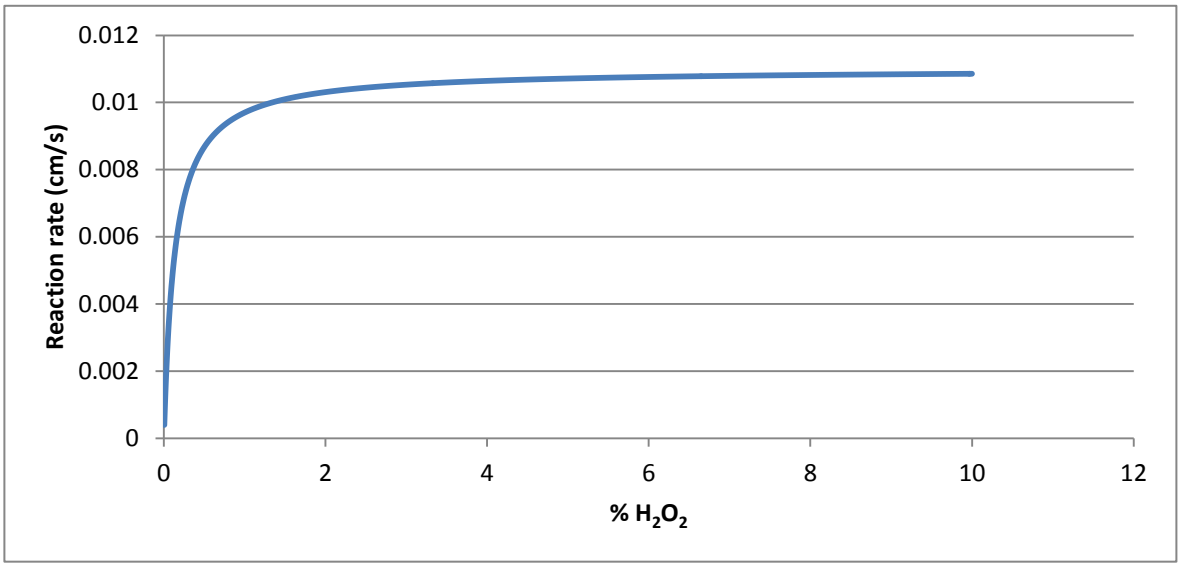

Figure 3: Michaelis-Menten relationship of catalase to hydrogen peroxide.

Discussion

These results clearly show the relationship between catalase and hydrogen peroxide. If there had been no reaction between catalase and hydrogen peroxide nothing could have been observed during this investigation. Therefore, the use of the evolution of oxygen as a proxy to observe the reaction was an effective means of measurement. The use of the proxy was a more crude way to approach this reaction measurement than if there had been some kind of machinery was used that could make more precise measurements. The opportunities for human error are varied and many. It was impossible for every disc to be soaked in the enzyme for the exact same amount of time resulting in some discs having a higher concentration of the enzyme than others. Although this was not addressed in this paper, a higher concentration of the enzyme can also lead to a higher rate of reaction, just as a higher concentration of substrate leads to a higher rate of reaction. This same idea applies to how fast the discs were placed at the bottom of their prospective beakers. As soon as the enzyme-soaked discs touched the solution they began reacting with any hydrogen peroxide available to them. This variance in time between the time the disc touched the solution and was released at the bottom increases chances for human error. Also, some of the discs would stick to the forceps when attempting to release them. This also increases time variances of reaction rate. The measurement of time was used with a hand-control stopwatch. This is not the most desired tool to use when trying to accurately measure precise times. Not starting the stopwatch at the precise time could cut

short the amount of time measured. Also, a finger could have slipped on the stop button and resulted in an excess amount of time added onto the measurement. On top of all these opportunities for human error is the fact that four individuals were involved in preparing the experiment and conducting it. This would not have been a potential problem if each person was assigned a single job to start and finish. But instead, for the sake of efficiency if not accuracy, jobs were exchanged between individuals with one person starting something and one or two other people finishing it. Each individual has his or her own habits of precision and this exchange across hands introduces even more confounding factors into the data. With all these imprecise practices involved in this experiment it was far from the most precise experiment and the results should not be relied upon heavily as scientific fact.

Because the V_{max} and K_M both were solved as negative numbers this implies that the relationship between catalase and hydrogen peroxide does not follow the Michaelis-Menten relationship. However, more precise investigations have been conducted and it had been known for many years that catalase and hydrogen peroxide do follow the Michaelis-Menten relationship (Peter Jones, 1968).

John Lattier and William Said also reported a V_{max} and K_M for purifies bovine liver catalase. A comparison of the data obtained from this investigation and Lattier's and Said's data can be seen in Figure 4. The Michaelis-Menten relationships for the data obtained in this investigation compared to the data obtained by Lattier and Said are distinctly different. Lattier's and Said's experimental conditions had less chance for human error and are probably more accurate.

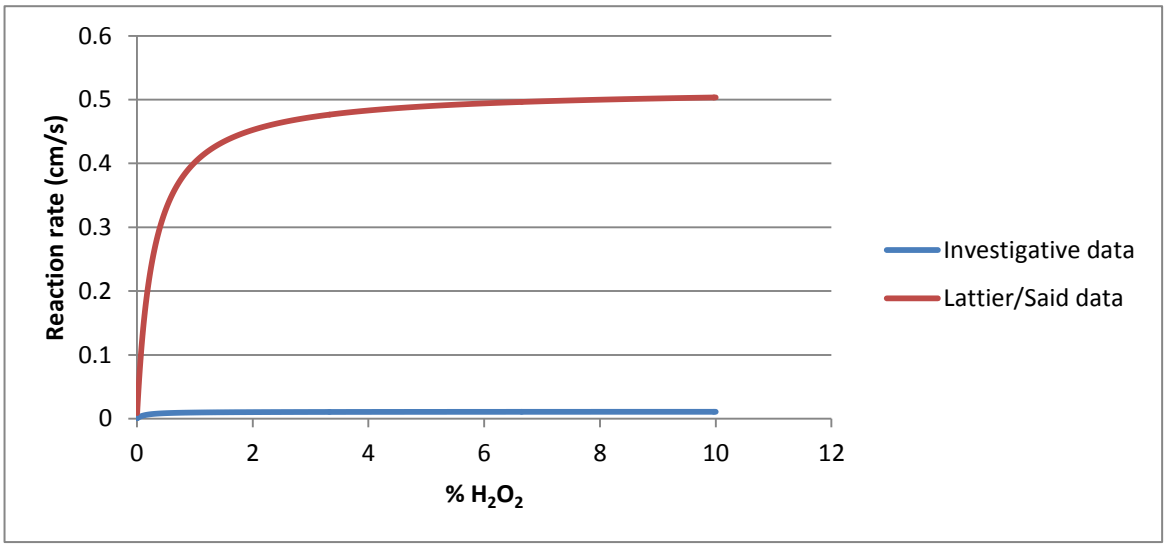

Figure 4: Comparison of Michaelis-Menten relationship data obtained in this investigation with Lattier/Said data.

If this experiment is ever repeated many things could be done to reduce the margin of human error. Individual investigators could work on single jobs to reduce the crossover of habitual precision each person has. The implication of precise machines would also decrease the chance for human error significantly. The fewer humans' hands that are directly involved in the procedure the less chance for human error. If there is a machine that can precisely administer the exact same amount of enzyme solution onto each disc the differing concentrations among discs would be reduced. Another machine could place the disc at the bottom of the beaker containing the enzyme. This machine could have a non-stick material that would prevent the discs from adhering to the clamp whenever placed in the solution. This machine could possibly also measure the exact amount of time it takes for the disc to rise to the surface. Any of these solutions would reduce future error. And a number of different approaches could be taken to investigate this relationship. A more complex procedure could measure the exact amount of oxygen evolution without the use of a proxy. The deletion of this crude intermediate would also make the data more precise.

Works Cited

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