Catalase Kinetics

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Kinetic studies on the activity of catalase conducted using a pressure gauge indicates that the enzyme has a V_{max} value of 0.0144, and K_m value of 0.00275. The catalase appears to be affected by fluctuating pH values, and operates most ideally at pH 9. Temperature studies determined the activation energy of the catalyzed reaction to be 0.00658 kJ/mol, and the reaction rate steadily increases with rising temperature. The combination of a Coomassie Protein Assay and a Ferrozine Assay determined that 1.56 iron atoms bind to each molecule of catalase, although this does not agree with the expected 4 iron atoms per catalase molecule.

Introduction

Catalase is an important biological enzyme responsible for the conversion of hydrogen peroxide to water and oxygen (Figure 1). Catalase is remarkably efficient, and one catalase enzyme can convert 40 million molecules of hydrogen peroxide to oxygen and water per second. This enzyme is necessary as a condition for survival, as the catalase breaks down hydrogen peroxide from accumulating to dangerous levels. Hydrogen peroxide is a strong oxidizer, and high levels in the body will induce cellular damage from oxidative stress.[1]

$$2H_2O_2(aq) \quad \text{----(catalase)--->} \quad 2H_2O\ (l) + O_2\ (g)$$

Figure 1. The decomposition of hydrogen peroxide to water and oxygen, as catalyzed by catalase.

The enzyme is a tetrameter comprised of subunits of 500 amino acids each, containing a heme (a prosthetic group containing an iron center). This structure is similar to that of the hemoglobin, also an important protein required for life.[2]

This experiment seeks to investigate the kinetics of catalase action, as well as factors that influence the rate of catalysis, including pH and the activation energy of the enzyme. Additionally, a protein assay used to determine the concentration of the catalase as well a ferrozine assay seeks to determine and detect the amount of enzyme-bound iron in catalase. The specific goals for the experiment are as follows:

(1) Investigate the enzyme activity of catalase through studying the decomposition of hydrogen peroxide to water and oxygen gas.
(2) Investigate the enzyme-bound iron content of catalase by using a modified ferrozine method.
(3) Perform a protein assay to determine the enzyme concentration of a given sample.
(4) Study the effects of reaction environment (temperature, pH) on the rate of the enzyme-catalyzed reaction.

Enzyme kinetics is governed by a series of equations. First, for a typical reaction aA + bB → cC + dD, the rate reaction is given by: rate = k $[A]^x$ $[B]^y$. In this equation, the k is the rate constant, and x and y indicates the order of the reaction in respect to each reactant. The variables x and y are different from the coefficients of a and b in the balanced equation. In order to measure the rate of the reaction empirically, one can go about

[1] Massachusetts Institute of Technology, *5.310 Laboratory Manual.*
[2] Boon, Downs, and Marcey. "Catalase: H_2O_2 Oxdioreductase,"
http://www.callutheran.edu/Academic_Programs/Departments/BioDev/omm/catalase/frames/cattx.ht
http://www.callutheran.edu/Academic_Programs/Departments/BioDev/omm/catalase/frames/cattx.ht
m#Topic2.

measuring the changes in pressure inside the reaction vessel over time. From approximating ideal conditions and using the PV=nRT equation, one can obtain the rate of the reaction being 1/RT d[P]/dt.[3]

Another important equation in enzyme kinetics is the Michaelis-Menten mechanism. The mechanism is summarized using the following schematic: (Figure 2)

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Figure 2. The Michaelis-Menton mechanism, with E and S standing for the enzyme and the substrate, respectively. ES is the intermediate complex, and P is the product of the reaction.

The Michaelis-Menton mechanism rests on three important assumptions. First, k-2 = 0, so the product will not revert back to the substrate. Second, the reaction proceeds at steady state (the concentration of the enzyme substrate complex (ES) is constant, and the concentration of the enzyme (E) is constant). Finally, the total amount of the enzyme is constant ($[E]_{total} = [E] + [ES]$). Through extended derivation of the original equation and these three assumptions, one may arrive at the Michaelis-Menton equation, which is presented in Figure 3.

$$\text{rate} = \frac{V_{max} \times [S]}{K_m + [S]}$$

Figure 3. The Michaelis-Menton equation demonstrating the relationship between reaction rate and the substrate concentration. V_{max} indicates the maximum rate of the reaction, while K_m is the concentration of the substrate when the initial rate is one half of its maximum rate. The rate calculated by this reaction is the initial rate of the reaction.

Taking the reciprocal of the Michaelis-Menton equation, one attains the Lineweaver-Burk plot, which uses graphical methods to obtain both V_{max} and K_m. The Lineweaver-Burk plot is given by Figure 4.

$$\frac{1}{rate} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

Figure 4. The Lineweaver-Burk equation, providing a linear plot when 1/rate and 1/[S] is graphed.

[3] Massachusetts Institute of Technology, *5.310 Laboratory Manual.*

The linear graph of 1/rate vs. 1/[S] in the Lineweaver-Burk plot provides several useful values. The slope of the line is K_m/V_{max}, the y-intercept is $1/V_{max}$, and the x-intercept is $-1/K_m$. Therefore, the Lineweaver-Burk plot provides easy access to the determinatino of these values in a kinetic measurement.

Finally, another important value in the study of enzyme kinetics is the value of E_a, or the activation energy of the reaction. The activation energy is the "energy bump" that is needed to be overcome to help a reaction get started and proceed from reactants to products without any other added energy. The catalyst helps promote the reaction by lowering the activation energy barrier. The relationship of the activation energy and the reaction rate is summarized by the Arrhenius equation (Figure 5).

$$k = A\, e^{\frac{-E_a}{RT}}$$

Figure 5. The Arrhenius equation, with E_a being the activation energy, given in kJ/mole, the R being the universal gas constant J/K × mol, the T being the absolute temperature in Kelvins, and A being the collision frequency factor.

Just like how the Lineweaver-Burk plot is a more useful depiction of the Michaelis-Menton equation, the ln k vs. 1/T plot can also be used to linearlize the relation and provide useful data. The ln k vs. 1/T plot for the activation energy is given in Figure 6.

$$\ln k = \frac{-E_a}{R}\frac{1}{T} + \ln A$$

Figure 6. The ln k vs. 1/T plot that linearizes the Arrhenius equation. This plot is especially useful because the slope of the resulting line is $-E_a/R$.

Two major laboratory techniques are introduced in this laborary experiment, including the measurement of rate and calorimetry experiments using a UV-Vis spectrometer. For the rate determination experiments, the reactants (catalase and hydrogen peroxide) are added to a sealed glass tube with a pressure sensor attached. During the reaction progress, the pressure inside the vessel is carefully monitored. Combined with the volume of the vessel and the reaction temperature, the oxygen pressure in the vessel can be converted back to the concentration of the oxygen in the vessel using the ideal gas equation. This information then yields the rate of the decomposition of hydrogen peroxide by catalase. Using this strategy, rate measurements are taken under three separate conditions – (1) varying the amount of hydrogen peroxide present in the vessel, (2) varying the pH of the phosphate buffer, and (3) varying the temperature of the reaction. These trials allow the experimenter to determine the K_m, V_{max}, E_a, and A of the kinetic reaction, as well as the optimal pH of catalase action.

The second half of the experiment relies on calorimetric analysis to determine the concentration of the enzyme present in an unknown sample of catalase, as well as the concentration of enzyme-bound iron in catalase.

Calorimetric analysis utilizes a UV-VIS spectrometer, and relies on two important observations in the two experiments. When the protein in the unknown sample of catalase binds to the Coomassie dye in the first assay, the absorbance of the dye shifts from 465 nm to 595 nm. In the second assay, when the ferrozine present forms a complex with ferrous iron, the complex absorbs at 562 nm. By exploiting these observations and measuring for absorbance at 595 nm and 562 nm, respectively, the researcher may determine the concentration of the protein in the first assay and the concentration of the enzyme-bound iron in the second assay.

Procedure

A sealed glass pressure tube that is connected to a pressure sensor was created and submerged in a water bath with an inserted thermometer. A stir bar was added to the pressure tube to ensure even stirring for every trial of the experiment. The pressure measurements were collected by the Logger Pro data-monitoring program.

To determine the K_m and the V_{max} of the kinetic reaction, six trial runs were run, which varies the concentration of the hydrogen peroxide while keeping the concentration of the catalase constant. The reaction conditions are summarized in Table 1, found in the Results section. The temperature of the water bath and the surrounding atmosphere was kept constant for these trials. To conduct each of these trials, the hydrogen peroxide and the phosphate buffer (the two should add up to 19.00 mL) were added to the pressure tube. The stir bar was activated, and the cap to the tube is screwed shut. The catalase (1.00 mL) was quickly added through an opening at the top of the tube, and the pressure sensor was promptly attached to the tube. At this time, the computer begins collecting pressure measurements in the tube for a total of 450 seconds. Each tube was carefully cleaned and dried at the conclusion of each trial, and the procedure was repeated for each trial.

To determine the optimal pH range of catalase activity, the phosphate buffer used in the experiment was altered using 2M HCl and 2M NaOH to pH values of 3.12, 5.13, 6.97, 8.94, and 10.98. Five trials were run using these buffers of varying pH values. To conduct each of these trials, 1.00 mL of hydrogen peroxide and 18.00 mL of the buffer was added together in the pressure tube, and the above procedure was repeated with 1.00 mL of catalase. The measurement procedure remains the same.

To determine the activation energy of catalase activity, the temperature of the water bath was altered using ice and hot water. Trials containing 18.00 mL of buffer, 1.00 mL of hydrogen peroxide, and 1.00 mL was run in the pressure tubes following the above procedure in water baths of 5, 17, 27, 32, and 41 degrees Celsius. To ensure that the temperature of the water bath is an accurate indicator of the temperature in the reaction vessel, the solution in the reaction vessel was allowed to reach thermal equilibrium at the beginning of each trial.

To determine the concentration of the protein in an unknown sample of catalase, a Coomassie Protein Assay was conducted. A standard curve was generated by creating 300 μL standard solutions containing varying volumes of bovine serum albumin solution and buffer (Table 4 in the Results section). Samples of 50 μL from each standard solution, three unknown samples from the catalase solution, and a blank (containing 40 mM Tris-HCl buffer, pH 8.2) were pipetted into Eppendorf tubes. A total of 1.5 mL of the Coomassie Plus reagent was added to each tube and mixed well. Each tube was allowed 10 minutes for the color to develop. Each sample was then transferred to 1.5 mL UV cuvettes, and the absorbance at 595 nm was measured using a UV-Vis spectrometer.

To determine the concentration of enzyme-bound iron in catalase solution, a Ferrozine Assay was conducted. A standard curve was generated by creating 300 μL standard solutions containing varying volumes of Fe AA (atomic absorption) standard and buffer (Table 5 in the Results section). A total of 100 μL of 4M methanesulfonic acid was added to each standard solution, as well as 300 μL of three unknown samples from the catalase solution. The tubes were placed into a 110 degrees Celsius sand bath for 40 minutes, and cooled for 5 minutes at room temperature prior to 2 minutes of centrifugation. A combination of solutions was added in the following order to each tube: 400 μL of 1% ascorbic acid, 400 μL of saturated ammonium acetate solution, 40 μL of 10N NaOH solution, and 100 μL of the iron complex mixture (10 mM ferrozine, 50 mg neocuproine, 3 drops of 6N Hcl in 25 mL Milli-Q water). The tubes were centrifuged for 10 minutes, and the solution was transferred into 1.5 mL UV cuvettes. Absorbance at 562 nm was measured using a UV-Vis spectrometer against a blank containing distilled water.

Appropriate safety procedures were undertaken throughout the entire course of the experiment, especially when working with strong oxdizers such as hydrogen peroxide or strong corrosives such as concentrated HCl and NaOH. All waste solutions were disposed per instructions in the fume hood, and personal protection equipment (gloves, googles) were worn for the entire duration of the experiment.

Results

Determination of V_{max} and K_m

Table 1. Experimental Data for the Determination of Parameters for the Kinetic Study of Catalase Action

Stock H_2O_2 (mL)	Phosphate buffer (mL)	Catalase (mL)	$[H_2O_2]$ (mol/L)	Calculated P_{max} (kPa)	Observed P_{max} (kPa)	Tangent slope (m, kPa/s)	Initial rate of rxn (1/s)	1/rate	1/ $[H_2O_2]$
0.15	18.85	1.00	0.00882	4.36	4.12	0.0470	0.0108	92.59	113.38
0.30	18.70	1.00	0.0176	8.70	8.70	0.116	0.0133	75.19	56.82
0.50	18.50	1.00	0.0294	14.53	14.62	0.185	0.0127	78.74	34.01

0.60	18.40	1.00	0.0353	17.44	17.78	0.239	0.0137	72.99	28.33
0.75	18.25	1.00	0.0441	21.79	22.49	0.264	0.0121	82.64	22.68
1.00	18.00	1.00	0.0588	29.05	30.13	0.433	0.0149	67.11	17.01

4% hydrogen peroxide solution = 40 g in 1000 g water = 1.18 M hydrogen peroxide stock. From this, it is possible to calculate the concentration of hydrogen peroxide in each solution. From that concentration, it is possible to calculate the moles of hydrogen peroxide in each solution. From Figure 1, we see that H_2O_2 : O_2 = 2 : 1, so moles O_2 is obtained by dividing moles H_2O_2 by 2. Then, the ideal gas equation (air volume of reaction vessel = 0.05 L) is used to determine P, which is P_{max} in kPa. This is compared against the observed P_{max}, which is simply the pressure in the reaction vessel at the completion of the chemical reaction subtracted by the initial pressure in the reaction vessel. The observed and calculated P_{max} matches up surprisingly well – showing that the theoretical hydrogen peroxide concentration in each reaction vessel matches closely with the actual concentration. The slope at the steepest point of the pressure vs. time graph for each trial was recorded and divided by the calculated P_{max} to yield the initial rate of the reaction. From that, the 1/rate and 1[H_2O_2] graph was generated and a linear regression curve found. The regression curve has an r-squared value of 0.61, y-intercept value of 69.54, and x-intercept value of -364.1. From this, it is possible to determine V_{max} as 0.0144, and K_m as 0.00275. (see Appendix for graph). Using LINEST, we get SD for slope as 0.00758, SD for y-intercept as 4.25, SD for y-values is 6.11. Therefore, slope = 0.191 $\pm$ 0.008, y-intercept = 69.54 $\pm$ 4.25.

To determine whether the system observes Michaelis-Menton kinetics, the observed empirical figures should follow Figure 3, the Michaelis-Menton equation, after plugging the empirically determined V_{max}, K_m, and [H_2O_2] back. The results (calculation omitted for brevity) generally confirm that this system does indeed follow Michaelis-Menten kinetics, although a complete correlation is compromised.

Determination pH on Catalase Activity

Reaction conditions: 18.00 mL of buffer of varying pH, 1.00 mL hydrogen peroxide, 1.00 catalase enzyme.

Table 2. Determination of pH on Catalase Activity

pH	Observed P_{max} (kPa)	Tangent slope (m, kPa/s)	Initial rate of rxn (1/s)
3.12	18.3	0.130	0.00710
5.13	19.3	0.137	0.00710
6.97	25.5	0.147	0.00577
8.94	30.1	0.360	0.0120
10.98	27.2	0.176	0.00647

The observed P_{max} was found by finding the difference in pressure of the reaction vessel before and after the experiment. The tangent slope is again the steepest part of the curve on the pressure vs. time graph. The initial rate of the reaction was found by dividng the tangent slope by the observed P_{max}. A graph of pH to rate of reaction is found in the Appendix.

Determination of Activation Energy

Reaction conditions: 18.00 mL of buffer of varying pH, 1.00 mL hydrogen peroxide, 1.00 catalase enzyme.

Table 3. Experimental Data for the Determination of Parameters for the Kinetic Study of Catalase Action

Temperature (degrees Celsius)	$[H_2O_2]$ (mol/L)	Calculated P_{max} (kPa)	Observed P_{max} (kPa)	Tangent slope (m, kPa/s)	Initial rate of rxn (1/s)	k	ln k	1/T
5	0.0588	29.05	25.1	0.266	0.0092	2.66	0.978	0.00360
17	0.0588	29.05	28.2	0.329	0.0117	3.38	1.218	0.00345
27	0.0588	29.05	30.1	0.358	0.0123	3.56	1.270	0.00333
32	0.0588	29.05	31.2	0.368	0.0127	3.67	1.300	0.00328
41	0.0588	29.05	33.4	0.459	0.0158	4.57	1.520	0.00319

The observed P_{max} was taken to be the difference in pressure between the final and initial positions. The calculated P_{max} and the concentration of hydrogen peroxide were taken directly from Table 1. The tangent slope of each run was obtained in the usual manner, by taking the steepest slope for each run. The initial rate of the reaction was obtained by dividing the tangent slope by the calculated P_{max}. After the initial rate was determined, the equation: rate = k $[H_2O_2]^2$ was used to determine k and ln k. This equation comes from the balanced equation of the overall chemical reaction given in Figure 1. Then, ln k vs. 1/T was plotted and a linear regression found. The regression has a r-squared value of 0.928, and a slope of -0.000791. Since this slope is equal to $-E_a/R$ (Figure 6), it is possible to determine the E_a for the system. The activation energy is 0.00658 kJ/mole, or 6.58 J/mole. Also, ln A is the y-intercept of the ln k vs. 1/T plot, so A = 1.00. Using LINEST, we get SD for slope as 0.000127, SD for y-intercept as 0.000161, SD for y-values is 0.0000493. Therefore, slope = -0.000791 $\pm$ 0.000127, y-intercept = 0.00436 $\pm$ 0.00016.

Determination of Protein Concentration

Table 4. Determination of Protein Concentration Standards and Unknowns

Volume of BSA (µL)	Volume of buffer (µL)	Final BSA Conc. (µg/mL)	Actual BSA Conc. (µg/mL)
300	0	2000	Exceeded instrument limit
375	125	1500	1300
325	325	1000	1012
175 of 1500 µg/mL	175	750	786
325 of 1000 µg/mL	325	500	552
325 of 500 µg/mL	325	250	234
325 of 250 µg/mL	325	125	104

Unknown 1	1181
Unknown 2	1330
Unknown 3	1219

A standard calibration curve was generated by using a non-linear fit of $A+Bx+Cx^2=0$ from the above data collected from the UV-VIS spectrometer. The r-squared value is 0.999 (graph see Appendix). The statistical data for the unknowns are average = 1243, SD = 77.42, 95% confidence interval = 1243 $\pm$ 192. The standard deviation of the measurements was found by using the equation: $\sigma = \sqrt{\frac{\Sigma(X-m)^2}{(n-1)}}$, where X = value of a trial, m = average of all trials, n = number of samples (three in this case). The equivalent can also be found using the STDEV command in Excel. The 95% confidence interval can be found using the equation: $\mathrm{CI} = m \pm 4.303\frac{\sigma}{\sqrt{n}}$, where σ = standard deviation, and n = number of samples. The 4.303 is the Z score of a 95% confidence interval and 2 degrees of freedom (3 samples) – this can be obtained by the TINV command in Excel.

Determination of Enzyme-Bound Iron

Table 5. Determination of Enzyme-Bound Iron in Ferrozine Assay

Volume of Fe AA Standard (100 µg/mL) (µL)	Volume of buffer (µL)	Final Fe Conc. (µg/mL)	Actual Fe Conc. (µg/mL)
0	300	0	0.400
3	297	1	1.270
6	294	2	2.566
12	288	4	4.290
18	282	6	6.002
24	276	8	7.933
30	2730	10	9.796
		Unknown 1	0.451
		Unknown 2	0.493
		Unknown 3	0.470

Statistical analysis of the unknowns: average = 0.471, SD = 0.021, 95% confidence interval 0.471 $\pm$ 0.052. The data points are plotted and a linear regression curve was found. The r-squared value is 0.999 (graph see Appendix).

Using the average concentrations of the protein and the iron, the ratio of moles ofiron per mole of catalase may be found. Catalase (one mole) = 230,731 grams, so 0.00539 umoles / mL (1243 ug/mL divided by 230,731 grams per mole). Fe (one mole) = 55.85 grams, so 0.00843 umoles / mL (0.471 ug/mL divided by 55.85 grams per mole). 0.00843 umoles / mL iron divided by 0.00539 umoles / mL catalase yields 1.56 iron per catalase.

Discussion

The purpose of this experiment is to empirically determine the kinetics of the catalase enzyme, by using Michealis-Menton kinetics model to determine K_m and V_{max}, Coomassie Protein Assay to determine the concentration of catalase in a given sample, and Ferrozine Assay to determine the concentration of enzyme-bound iron. Additionally, the effects of pH on catalase activity and the activation energy of catalase was also determined.

Determination of V_{max} and K_m

Although the calculated and the observed P_{max} values matched up pretty well, the linear regression Michaelis-Menton plot did not fit very well. The r-squared value of 0.61 indicates that there was a presence of significant experimental error. These errors may have been caused by (1) uneven stirring of the stir bar in the reaction vessel, (2) inaccuracies in the measurement of reagents prior to beginning each trial, and (3) differences in the volume of catalase added due to accidental overflow of the enzyme while injecting it into the reaction vessel. The system was determined to obey the Michaelis-Menten kinetic mechanism, although a complete correlation was compromised likely due to the same factors pointed out here. The low r-squared value in the regression line indicates that there was error present in the data collection phase, which may have caused the final rate calculations to be not completely accurate. The common value for K_m of catalase in literature is 25 mmol[4], while our obtained K_m value was 2.75 mmol, which is about an order of magnitude too small. Again, the discrepency here can be attributed to the factors of error pointed out above. As such, extra care should be demonstrated if the experiment was to occur again, to yield better results for V_{max} and K_m.

Determination pH on Catalase Activity

From the pH assay conducted, it appears that catalase operates the best at conditions around pH 9. pH affects the action of catalase by lowering or raising the initial rate, so that the enzyme either becomes less efficient or more efficient. At a molecular level, pH affects enzymes by altering the state of ionization on the amino acids on the active sites of the enzymes. If the state of ionization is changed, this affects the overall 3-D structure of the enzyme, and decreased binding to substrate may occur. At extreme pH values, the protein may be so affected that it ceases to be functional because it can no longer recognize the substrate. Again, it appears that there is more experimental error in this part of the experiment, as the optimal pH that catalase operates under should be pH 7[5]. There were difficulties associated with the pressure sensor during this part of the experiment, and the pH 7 trial was repeated for three times until a satisfactory measurement was obtained. However, the results indicate that the runs were still not free from experimental error, despite trying for several times. pH

[4] Bradford, B. U. (2007), "Role of peroxisomes in the swift increase in alcohol metabolism." *Journal of Gastroenterology and Hepatology*, *22*: S28–S30. doi: 10.1111/j.1440-1746.2006.04641.x

[5] Worthington Biochemical Corporation, "Effects of pH: Introduction to Enzymes," http://www.worthington-biochem.com/introbiochem/effectspH.html.

solutions were made properly and verified several times, so the error was probably caused by issues with the reaction vessel.

Determination of Activation Energy

Catalase, being a catalyst to the decomposition of hydrogen peroxide, lowers the activation energy to the reaction so it makes the reaction easier to proceed. The uncatalyzed reaction of hydrogen peroxide decomposition generally has a value of 75 kJ/mol, whereas catalase could lower the value to below 8 kJ/mol.[6] Physiologically, catalysts are extremely important, as they greatly facilitate the speed that common chemical reactions occur. Without catalysts, essential reactions would take days or months to occur, and toxic waste would accumulate quickly in the body. Therefore, it is vital that catalase lower the activation energy of the reaction. At the same time, however, the result obtained in this experiment for the activation energy is only 0.00658 kJ/mol, which appears to be rather small. Given the uncatalyzed reaction has an activation energy of 75 kJ/mol, it does not seem very likely that the catalyzed activation energy is quite so small.

Temperature studies indicate that the rate of the reaction increased as the temperature was raised. This makes sense because molecules collide at a greater frequency and with more force at higher temperatures. Thus, substrates and enzymes can locate each other easier in the system, speeding up the rate of the reaction. Therefore, one expects reaction rates to rise when the temperature is increased, although it is important to note that after a certain point this will no longer be true, as sustained high temperatures will begin to denature the catalyst, lowering the reaction rate dramatically. This is especially of note in this experiment, as the enzyme appears to work even at high temperatures such as 41 degrees Celsius. Generally, it is expected that the optimal temperature range of catalase should be at physiological temperatures, and above a certain point enzyme activity will diminish due to heat. The results of this experiment indicate that this threshold may yet be higher for catalase.

Determination of Protein Concentration

The extremely high r-squared value when fitted with a non-linear curve indicate that the experiment was conducted successfully, as there is a high degree of correlation. Sources of possible systematic error include (1) improperly calibrated pipettes, (2) solutions that are not at the right molarity, and (3) improper pipetting techniques. Sources of possible random error include (1) mixing up samples, (2) pipetting wrong reagents or missing reagents, and (3) random contamination of tubes or beakers. Given the high degree of correlation in the results, it is fortunate that these factors of error were generally not present.

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[6] Purdue University, "The Catalytic Decomposition of Hydrogen Peroxide," http://chemed.chem.purdue.edu/demos/demosheets/19.6.html.

Determination of Enzyme-Bound Iron

Through the comparison of the iron concentration and the protein concentration, the conclusion of 1.56 iron to one catalase was reached. Quite clearly, the answer should be 4 iron atoms to one catalase, but this low value may be attributed to the fact that the catalase solution used for the two experiments were completely different, since the experiments were conducted over a week apart. However, the most likely reason for the discrepency may be that this is a very simplistic way to determine the number of iron atoms bound to catalase, and a much better method would be to use X-ray diffraction, spectroscopy, or protein structure modeling to visualize the structure of the enzyme and the binding sites for the iron specifically. Non-specific binding during both assays could have shifted the final result in either direction, and as such, these assays only provide a rough figure of the number of bound iron atoms, but not the definitive value.

Conclusion

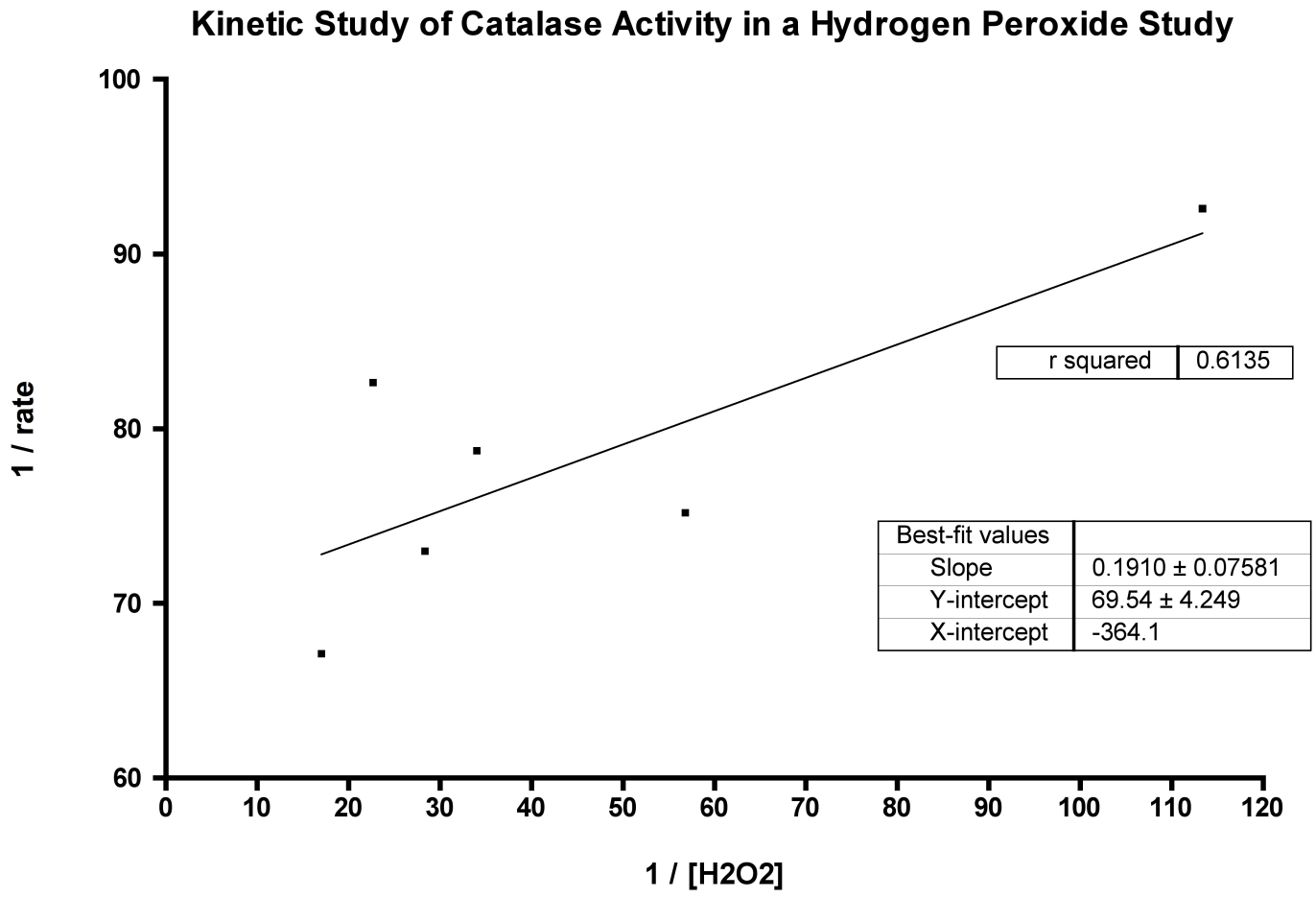
Studies using the enzyme catalase shows that the catalyst follows the Michealis-Menton kinetic model, and the reaction rate of the catalyst is influenced by factors such as concentration of substrate, temperature, and pH. The Coommassie protein assay and the ferrozine assay indicate that multiple iron atoms are associated with each catalase molecule, although the reported 1.56 iron atoms per catalase is not in agreement with the expected 4 iron atoms per catalase.

References

-Massachusetts Institute of Technology, *5.310 Laboratory Manual*

-World Protein Data Bank, 8CAT, http://www.rcsb.org/pdb/explore.do?structureId=8cat.

Appendix


Kinetic Study of Catalase Activity in a Hydrogen Peroxide Study
1 / rate
1 / [H2O2]
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Best-fit values
Slope
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Y-intercept
69.54 ± 4.249
X-intercept
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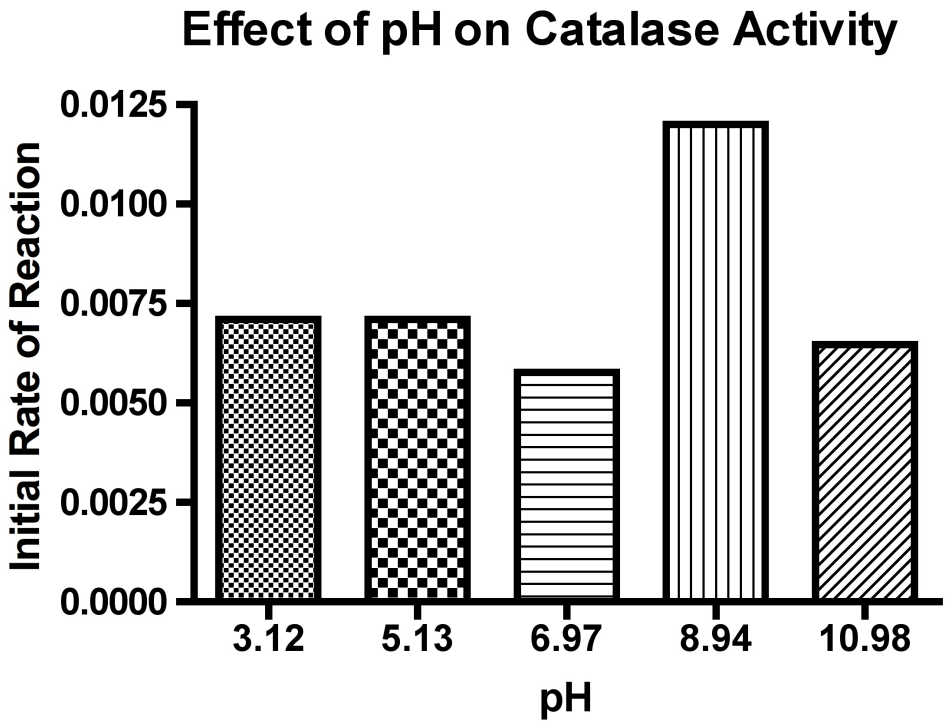
Effect of pH on Catalase Activity
Initial Rate of Reaction
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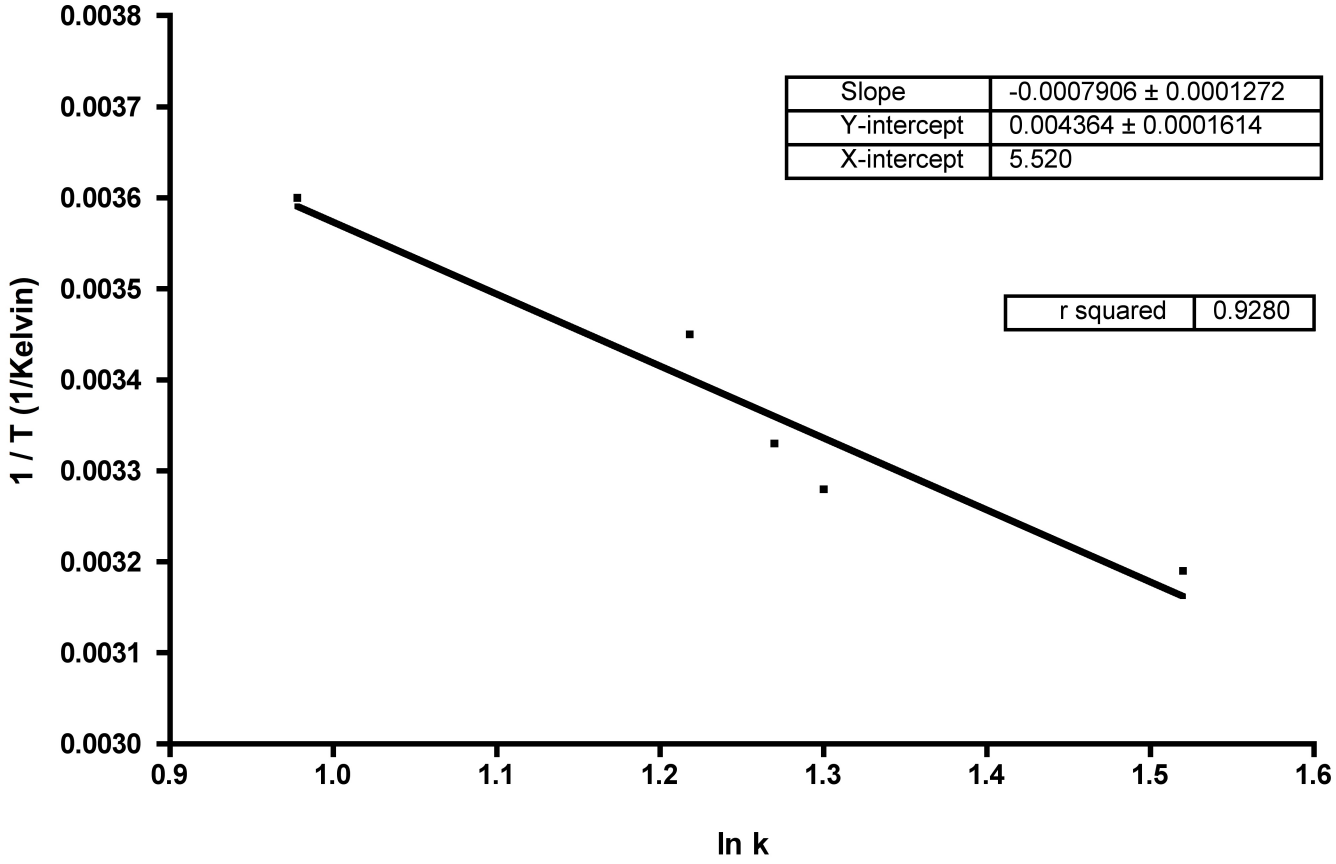
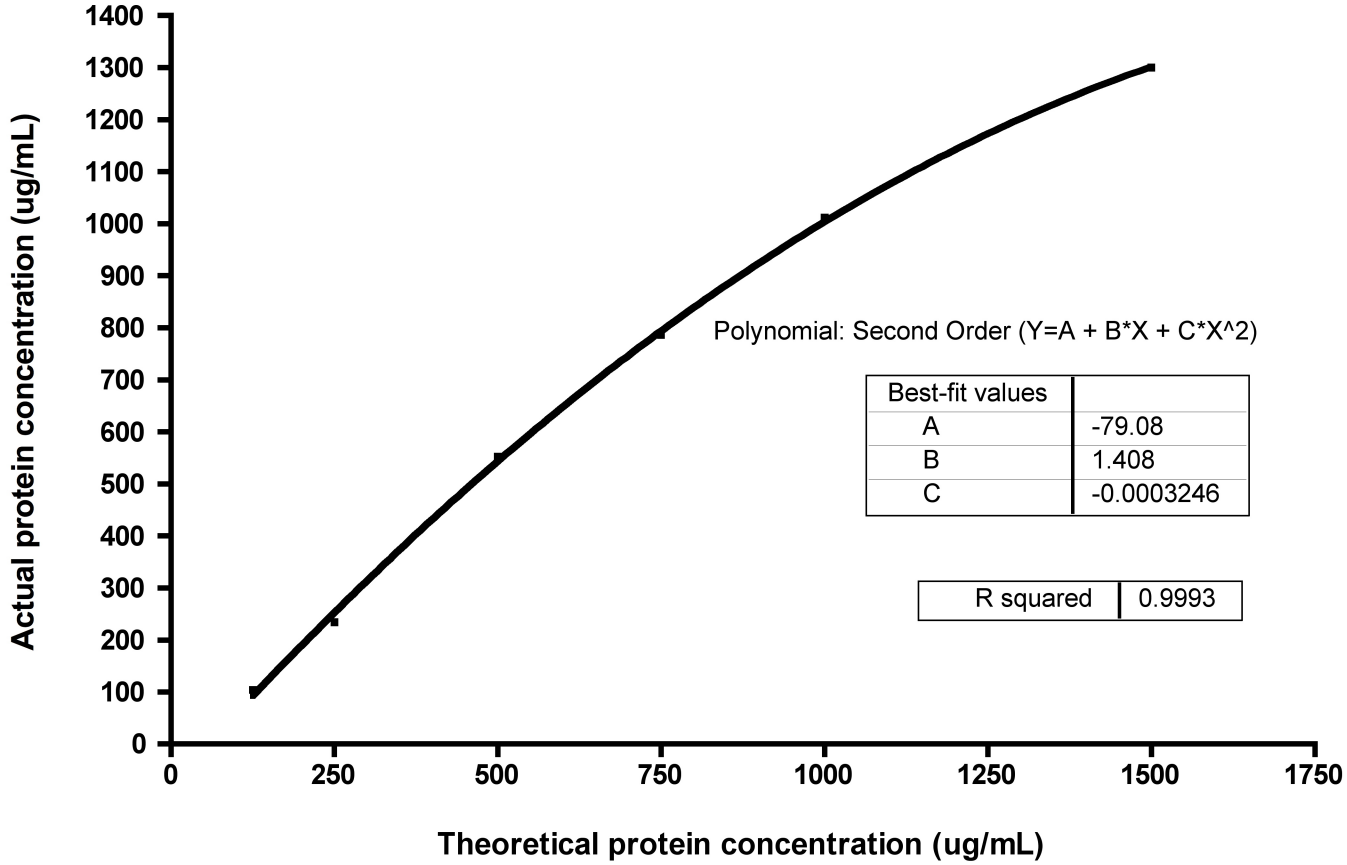
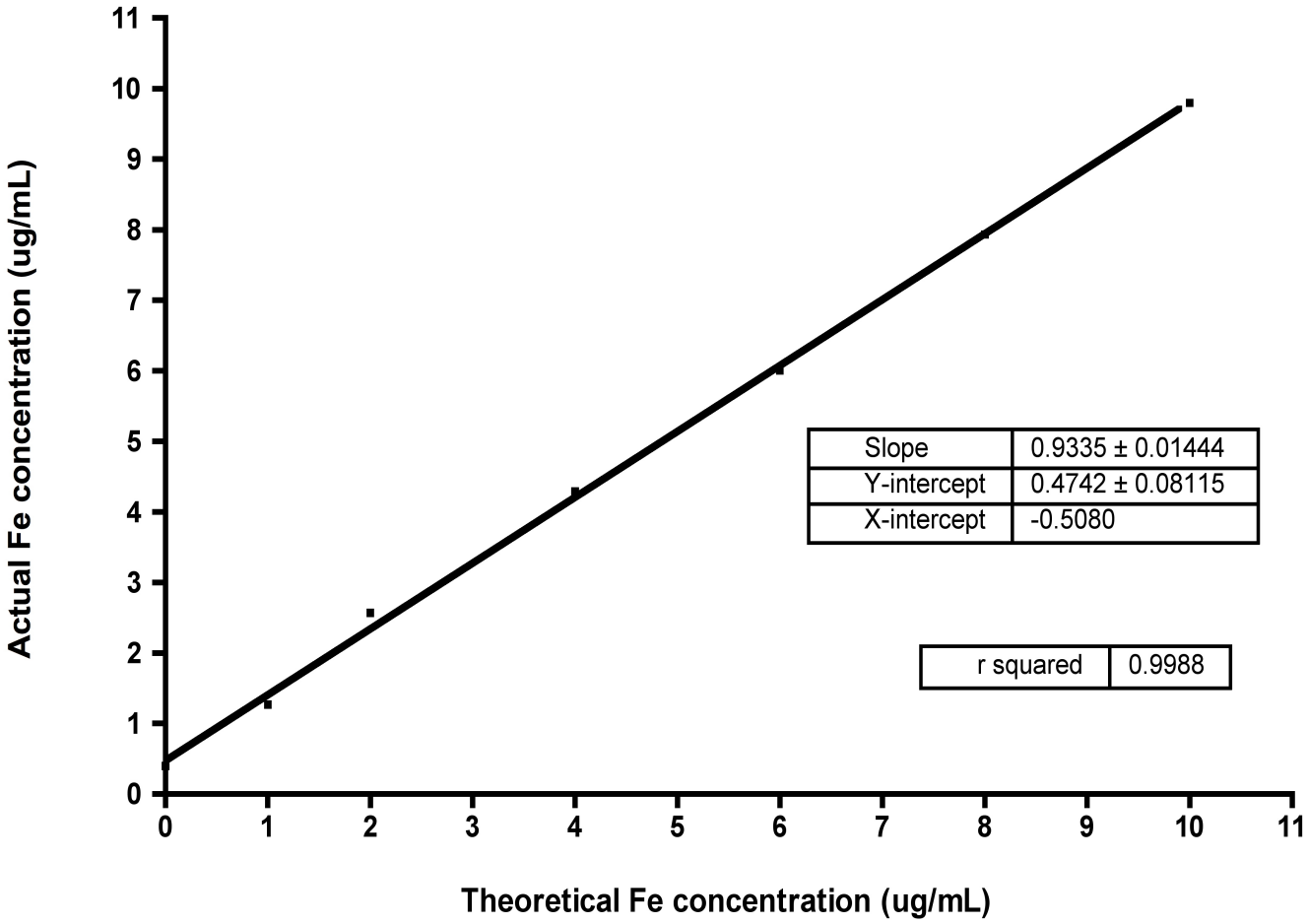
3.12
5.13
6.97
8.94
10.98

Determination of Activation Energy of Catalase Reaction



Standard Curve of Enzyme Concentration in Catalase Sample




Standard Curve of Iron in Ferrozine Assay
Actual Fe concentration (ug/mL)
Theoretical Fe concentration (ug/mL)
0
1
2
3
4
5
6
7
8
9
10
11
Slope
0.9335 ± 0.01444
Y-intercept
0.4742 ± 0.08115
X-intercept
-0.5080
r squared
0.9988