The Beefy Catalase

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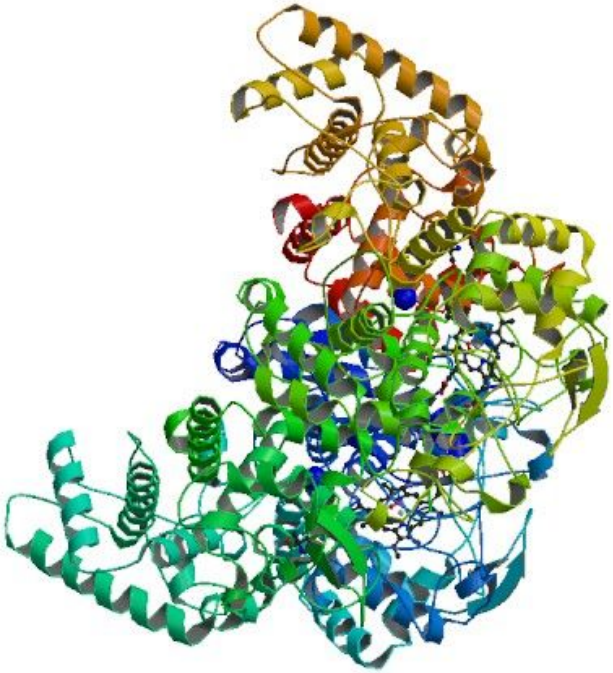


Size of Catalase: 250,000 g/mol

Purpose of Project

- Assay to measure the hydrogen peroxide substrate remaining after the action of catalase
- Analyze the activity of the enzyme catalase in different samples
    - Evaluate which tissue samples contain the highest amount of catalase and why
- Observe the stability of catalase over multiple days
    - Determine how the structure allows for stability

General Catalase Information

- Produced by aerobic organisms
- Optimal temperatures vary by organisms:
  - Humans: 37° C
  - Yeast: 40° C
  - Archaebacteria: 90° C
- Commonly found in the liver
  - Located in the **peroxisome** organelle

History of Catalase

- Louis Jacques Thenard discovered that there is some substance that breaks down hydrogen peroxide in the early nineteenth century
- 1900: Catalase discovered as substance that degrades H_2O_2
  - Oscar Loew coined the term “catalase”
  - Discovered that catalase was found in many plants and animals
- 1937: James B. Sumner and Alexander Dounce successfully create catalase from crystallized bovine liver

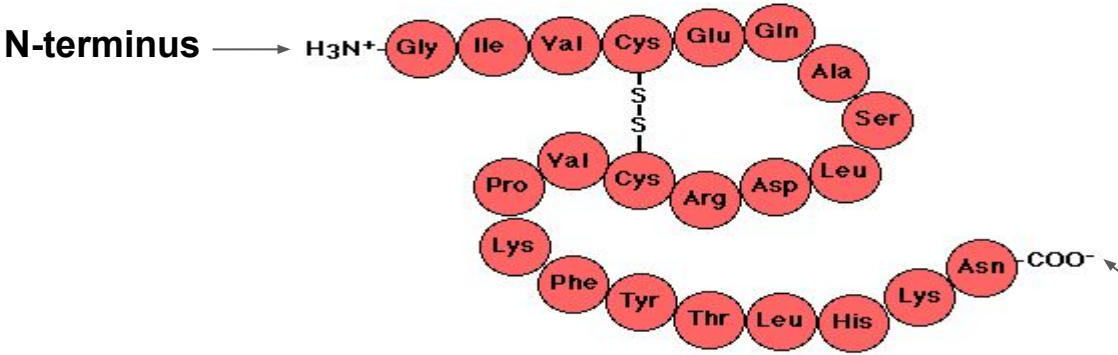
The Role of Catalase

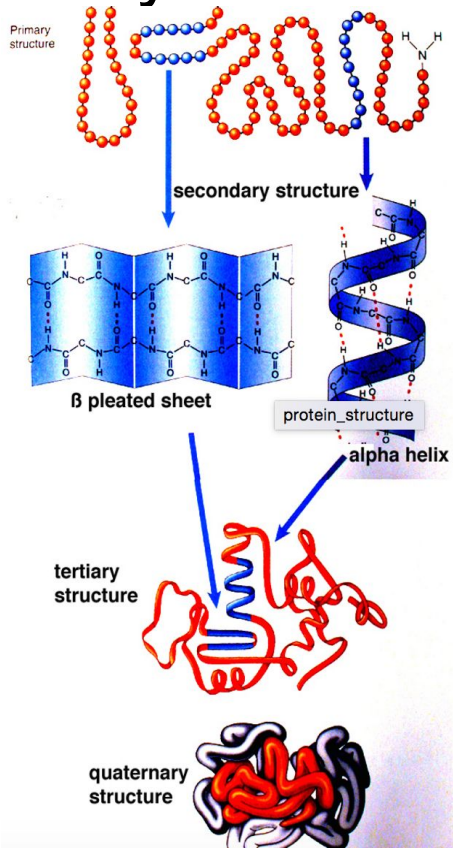
- Protects cell from oxidative damage by hydrogen peroxide (H_2O_2)
- Found in plants and animals
- Facilitates decomposition of H_2O_2 into water (H_2O) and oxygen (O_2)

$$2\ H_2O_2 \rightarrow O_2 + 2\ H_2O$$

Structure of Catalase: Primary and Secondary

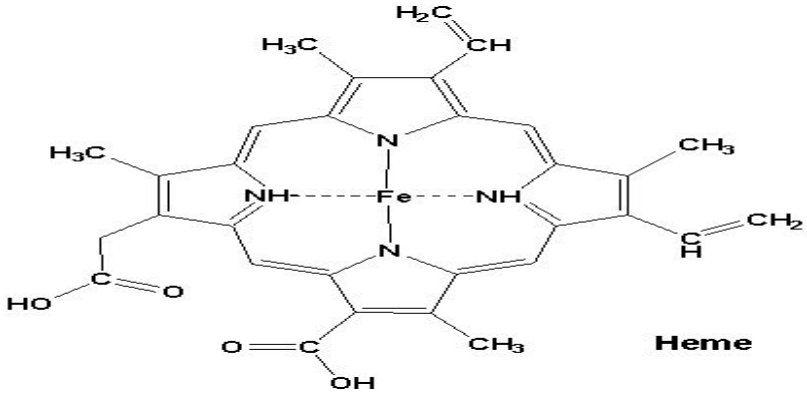
- Primary: string of 500 amino acids linked by peptide bonds
- Secondary: consists of alpha helixes and beta sheets

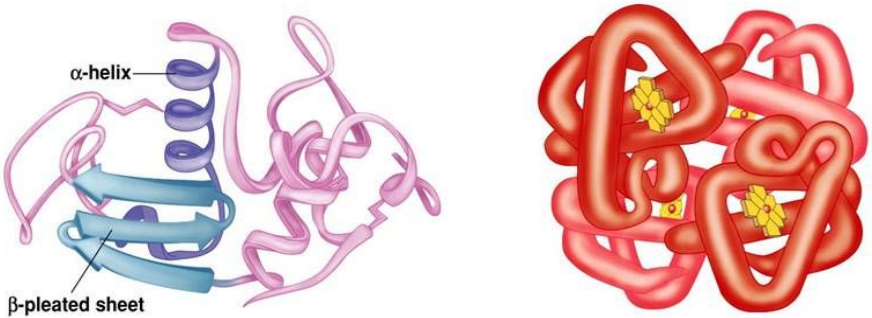




Structure of Catalase: Tertiary and Quaternary

- Tertiary: each unit consists of channel with **porphyrin heme**
  - **Porphyrin Ring**: water-soluble biological pigment
  - **Heme**: cofactor consisting of Fe^{+3}
  - Porphyrin kelases (grabs) the iron molecule
- Quaternary: linkage between protein structures is highly rigid

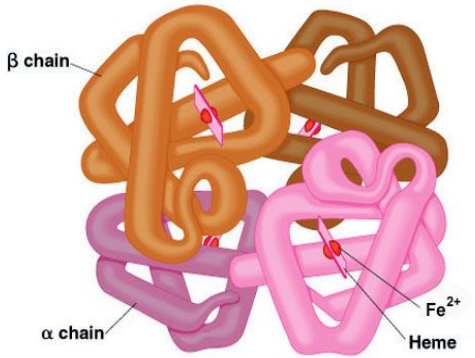




How Catalase Works

- H_2O_2 enters the active site
- Turns H_2O_2 into water and oxygen in two steps:

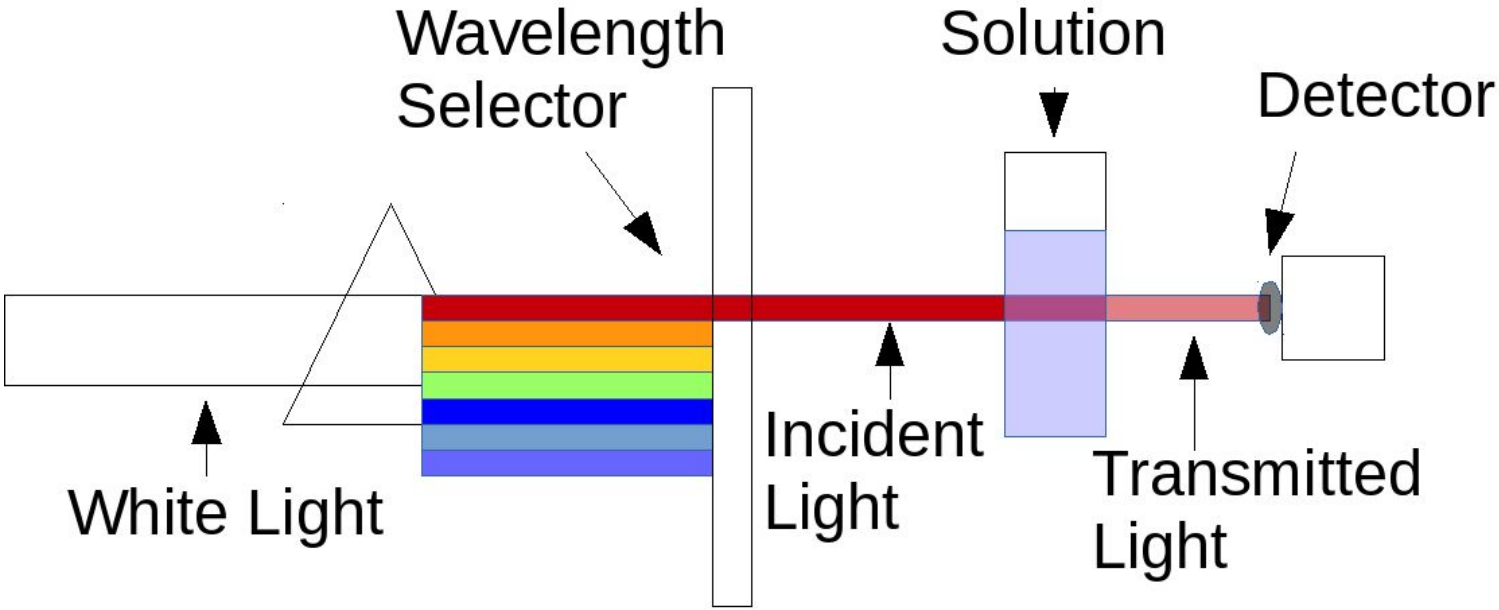
1. $H_2O_2 + Fe^{+3}$ enzyme >>>> $H_2O + Fe^{+4}$ enzyme
2. $H_2O_2 + Fe^{+4}$ enzyme >>>> $H_2O + Fe^{+3}$ enzyme + O_2



Spectrophotometer

- Method used to measure amount of light absorbed by a chemical substance
- Every chemical compound absorbs, transmits, or reflects light
- Transmittance: Fraction of light that passes through the sample
- Absorbance: Amount of photons (light) that are absorbed

Spectrophotometer



UV-VIS

220-800 nm

Spec 20

400-700 nm

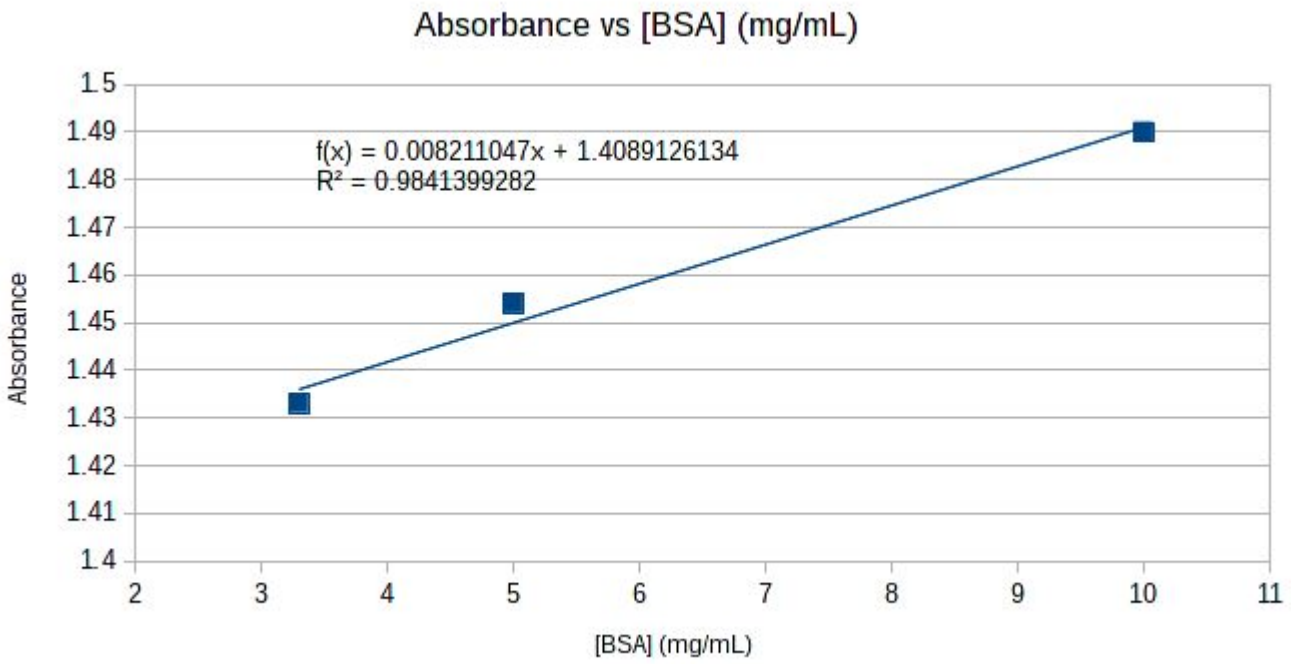
Protein Quantitation of Bovine Serum Albumin (BSA)

- Set up 5 samples with cuvettes, 1 blank (3 mL dH_2O) and 4 other cuvettes of dilutions.
- 3 mL BSA in cuvette 1, 2 mL in 2, 1.5 mL in 3, and 1 mL in 4.
- Add enough dH_2O so 1,2,3, and 4 are a total volume of 3 mL.
- Add 3 mL Bradford Reagent to each tube, and wait for 5 minutes.
- Blank spec 20 at A_{595} take A_{595} of cuvettes 1-4.

Given Data:

A_{595}	**[BSA] mg/mL**
1.49	10
~~1.503~~	~~6.7~~
1.454	5
1.433	3.3

Finding unknown concentration of BSA



Given 1 mL of unknown

2 mL Bradford Reagent

Two cuvettes: 1 blank

(1 mL Bradford + 2mL water)

1 solution (1 mL Bradford + 1 mL BSA + 1 mL water)

Take the A_{595}

Extract Preparation

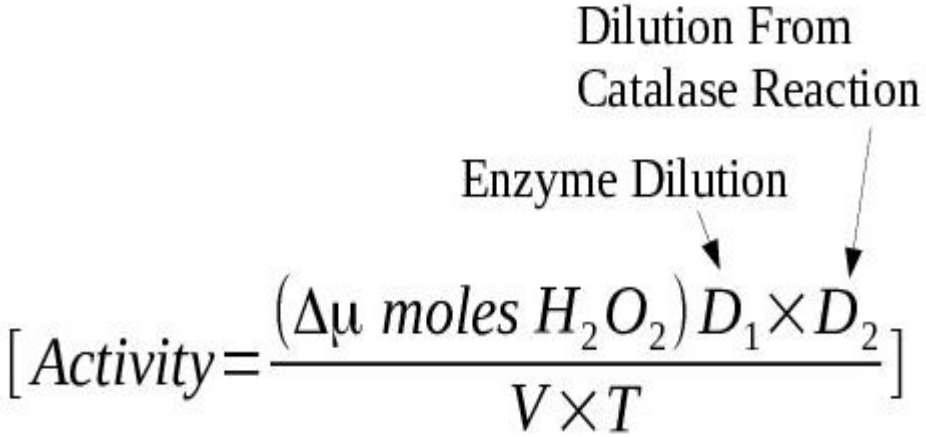
- Centrifuge (1)
- Chilled or frozen tissue (enough for 1-2g)
- Small beaker (1)
- Chilled mortar and pestle (1)
- PB (10 mL)
- Plastic tubes (4)
- Plastic pipet (2)
- Glass cuvette (1)
- Parafilm (1 strip)

1. Measure 1-2 grams of respective tissue
2. Place tissue in chilled mortar and pestle
3. Measure 10 mL of PB and pour into mortar
4. Smash and mix tissue with PB until a consistent, smooth liquid forms
5. Pipet 1.5 mL of liquid into plastic tube; Repeat for other three tubes
6. Centrifuge mixture for 10 minutes
7. Use a new pipet supernatant from each tube into glass cuvette
8. Parafilm and store in refrigerator overnight

How the Assay works

1. Prepare three dilutions for both the catalase and crude extract
2. Prepare three Blanks, two reaction tubes and five Stops
3. Transfer 6 λ of the third dilution of both catalase and crude extract into Blank
4. Wait four minutes for catalase to react
5. Transfer 100 λ of each Blank into the Stops
6. Set up 6 cuvettes with 1 mL color reagent (one Blank with Color Reagent + five stops)
7. Transfer 100 λ of stops into the cuvettes, wait 15 minutes. Check A_{520}

Calculating Catalase and Protein Activity



Linear Regression Report

Page/Date/Time 1 9/30/2015 8:17:25 AM
Database
Y = C1 X = C2

Linear Regression Plot Section

9-30-15

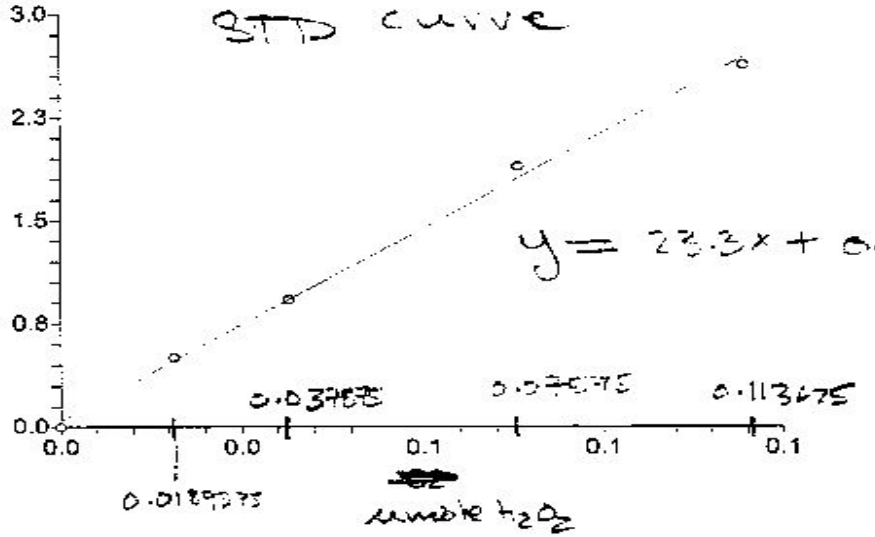
~~C1 vs C2~~

STD curve

Set up exactly like Tech Bulletin

$y = 23.3x + 0.0433$

1.520



Calculation Walkthrough

Given this set of data (Absorbances):

Blank 1: .88464

Blank 2: .85779

Blank 3: .8492

Crude Extract: .86285

Purified Catalase: .87840

H_2O_2 : .71019/.0436 = 16.28 mg/mL

Bradford: .6618

Calculation Walkthrough (cont.)

Change in micromole H_2O_2 :

Blank 1: .88464 / 23.3 = .037967

Blank 2: .85779 (ignored)

Blank 3: .8492 (ignored)

Crude Extract: .86285 / 23.3 = .037032

Purified Catalase: .87840 / 23.3 = .0377

H_2O_2 : .71019/.0436 = 16.28 mg/mL

Bradford: .6618

Calculation Walkthrough (cont.)

$$Catalase: \frac{(.037967 - .0377)(10{,}000)(100)}{.3(4)} \approx 223.176 \ \frac{\mu\text{mole } H_2O_2}{\text{mL min}}$$

$$.6618 \approx .0076x + .5217$$
$$x \approx 18.4342$$

$$\frac{223.176}{2.2} \approx 101.444 \ \frac{\mu\text{mole } H_2O_2}{\text{mL min mg}}$$

$$\frac{779.167}{18.4342} \approx 42.276 \ \frac{\mu\text{mole } H_2O_2}{\text{mL min mg}}$$

$$Crude\ Extract: \frac{(.037967 - .037032)(10{,}000)(100)}{.3(4)} \approx 779.328 \ \frac{\mu\text{mole } H_2O_2}{\text{mL min}}$$

$$101.136/42.276 \approx 2.4$$

Tissue Data

Tissue	Volume (mL)	Total Protein (mg)	Activity (units)	Total Activity (units * ml)	Specific Activity (units / mg)	Total Activity / Total Protein	Total Activity Per Wet Mass of Tissue
Chicken Liver	4.00	8.22	3080.59	12322.37	374.89	1499.54	10853.53
Beef Liver*	4.00	16.39	3048.64	12194.56	185.98	743.93	8486.12
Avocado*	4.00	15.54	2524.86	10099.42	162.43	649.72	5919.94
Potato*	4.00	5.92	1531.66	6126.62	258.86	1035.46	4816.53
Beef Heart*	4.00	5.69	378.04	1512.16	66.46	265.86	1213.61

Bradford Equation: y = .101x + .4122

*Only had two trials

Beef Liver Extended Lifetime

Tissue	Volume (mL)	Total Protein (mg)	Activity (units)	Total Activity (units * ml)	Specific Activity (units / mg)	Total Activity / Total Protein	Activity Per Wet Mass of Tissue	H2O2 mg/mL
Beef Liver Day 1	4	7.49	899.50	3598.00	120.17	480.66	477.79	15.05
Beef Liver Day 4	4	7.49	2320.46	9281.84	309.99	1239.97	1232.57	14.80
Beef Liver Day 11	4	7.49	2244.64	8978.56	299.86	1199.453151	1192.30	14.58

How is an extended lifetime possible?

1. Catalase activity increases as temperature gets lower
2. Low temperatures and high concentrations of H_2O_2 have low catalase activity (when low temp wouldn't favor)
3. At a specified temperature, catalase activity decreases as H_2O_2 concentration increases

TABLE I.

Relative Catalase Activity.

Temperature.	H_2O_2 concentration.				
	0.36 N	0.54 N	0.72 N	0.90 N	1.08 N
°C.					
0	55.5	76.0	40.5	29.8	13.0
8		**100.0**	88.2	61.4	62.8
19.4	68.2	73.5	66.6	57.7	49.5
30	46.5	48.0	44.7	42.7	38.5
	0.36 N	0.72 N	1.08 N	1.44 N	1.80 N
3	**100.0**	82.6			49.0
11.5	95.5	70.9	53.0		
15.8	90.7		49.1	50.4	37.3
19.8	73.4	55.6	49.5	42.6	33.9
24.5	56.9	49.6	42.6	39.6	32.6
	0.37 N	0.55 N	0.74 N		
0		41.7	21.4		
10	**100.0**	82.5	56.0		
16.1	96.2	75.0	59.5		
20.2	71.5	60.5	52.4		
24.7	59.8	53.9	49.5		
29.5	49.0	44.5	41.2		
	0.22 N	0.36 N	0.50 N		
15.1		97.0	**100.0**		
20		78.0	70.0		
25.4	57.9	58.6	59.0		
30	48.2	44.8	44.8		

Conclusions

- Catalase stays stable over a long period of time as long as temperature stays at optimal low temperature
- What went wrong:
  - Pipetting
  - Maintaining a consistent H_2O_2 values across multiple days for a single tissue sample
  - The results would differ because of the variant H_2O_2.

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Thank you! Any questions?