応用化学実験第一

後藤・神谷研究室

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Contents

FIRST EXPERIMENT

Conducted on November 16, Wednesday

1 Inactivation of Alkaline Phosphatase: Evaluation of Enzyme Thermal Stability

1.1 Introduction

Enzymes are biocatalysts that are now widely used in various fields. One of the most basic and useful analytical methods for determining the catalytic properties of enzymes is kinetic analysis. By analyzing enzymatic reactions along a time axis, one can infer the reaction mechanism of newly isolated enzymes or enzymes in a specific reaction field. In this experiment, the Michaelis constant (Km) and maximum reaction rate (Vmax) were determined by first rate analysis using the hydrolysis reaction of phosphate ester by alkaline phosphatase (BAP) from Escherichia coli as a model. The same analysis will be conducted using alkaline phosphatase (CIAP) from small intestine of calf to study the differences in enzyme characteristics depending on the origin of the enzyme. In addition, we will deepen our understanding of enzyme inactivation and handling as a protein.

In Experiment 1, the thermal stability of enzymes BAP and CIAP is evaluated.

1.1.1 Materials

Reagents

- (a) Buffer solution: 10 mM Tris-HCI buffer (pH 8.0) 10 ml
- (b) Product solution: 0.10 mM p-nitroferol (p-NP) solution (pH 8.0) 1.0 ml
- (c) Substrate solution: 0.25 mM p-nitrophenyl phosphate (p-NPP) solution (pH 8.0) 6.0 ml

- (d) Enzyme solution 1: 0.10 mg/ml BAP solution (pH 8.0) 0.10 ml
- (e) Enzyme solution 2: 1.1 ug/ml CIAP solution (pH 8.0) 0.10 ml
- (f) Stopping solution: 1M sodium hydroxide solution 6.0 ml

Glasswares

- 96-well plate
- Eppendorf microtubes
- Two 10-100 ul adjustable micropipettes
- One 100-1000 ul adjustable micropipettes

Instruments

- Microplate Reader

1.2 Methodology

1.2.1 Preparation of product calibration curve

(1) 100 pl of Stopping Solution (f) were added to each well (C1-C6) of a 96-well plastic plate.
(2) 0.2 ml of aqueous product p-NP solution at various concentrations of [p-NP]=2.5, 5.0, 15, 25, 50 uM was prepared using buffer (a) and product solution (b).
(3) 100 ul of buffer solution (a) was added to the well of C1.
(4) 100 pl of the product solution of (2) was added to lanes C2 to C6.

1.2.2 Evaluation of thermal stability of enzyme

(1) To each well ((A, B)×(1-12)) of a 96-well plastic plate, 100 ul of stopping solution (f) was added.
(2) 0.18 ml of buffer (a) was added to each of four microtubes.
(3) Enzyme 1 (BAP) solution (d) was added to two of the tubes, 20ul each.
(4)Enzyme 2 (CIAP) solution (e) was added to the remaining two tubes of (2) by 20ul each.
(5) One bottle each of (3) and (4) was incubated at 65°C for 30 min using a thermostatic incubator. The remaining one bottle each was allowed to stand at room temperature.
(6) Enzyme activity assay was performed using the enzyme solution of (5).

First, 1.0 ml of substrate solution (C) was measured in each of four microtubes. 100 ul of substrate solution (c) in the tube was added to the well of A1. Next, 0.10 ml of BAP, 25C solution out of the enzyme solution in (5) was added and the Eppendorf tube was quickly inverted to homogenize the solution and start the reaction. 100 ul of the reaction solution was added sequentially from A2 to A6, sampling every minute for 5 minutes.

Enzyme activity was measured using the other enzyme solutions in (5) in the same manner as in (6): BAP, 65°C (A7-A12); CIAP, 25°C (B1-B6); CIAP, 65°C (B7-B12).

1.3 Results

The results of the experiment were analyzed and are shown as follows.

1.3.1 Background calibration

The background calibration curve was conducted using solution (b) Product solution: 0.10 mM p-nitroferol (p-NP).

Well position	p-NP concentration (uM)	Average absorbance (410 nm)	Corrected absorbance (410 nm)
C2	2.5	0.0449	0.0085
C3	5	0.0523	0.0159
C4	15	0.1168	0.0804
C5	25	0.1731	0.1367
C6	50	0.3103	0.2739

	Background correction absorbance (410 nm)
C1	0.0364

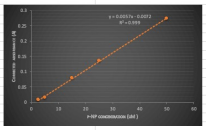


Figure 1: Background calibration

Here, the calibration is conducted according to the linear equation 1.

$$y = ax - b \tag{1}$$

In which x represents the concentration of p-NP, and y represents the corrected absorbance. The values of a and b constants are as follows:

$$a = 0.0057$$

$$b = 0.0072$$

Therefore, the concentration of p-NP can be calculated from the corrected absorbance measured by using equation 2.

$$x = \frac{y + b}{a} \tag{2}$$

1.3.2 Enzyme BAP

These are the graphs for the thermal stability of enzyme BAP. The concentration of p-NP was calculated by using equation 2.

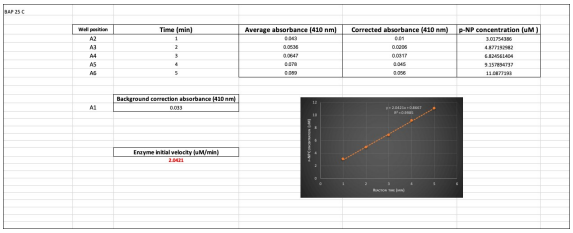


Figure 2: BAP performance at 25°C

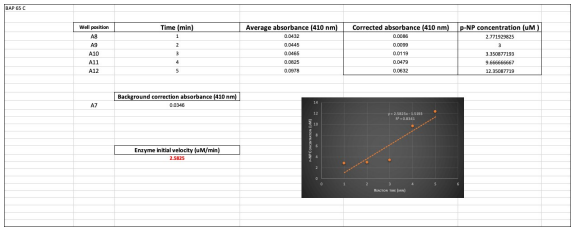


Figure 3: BAP performance at 65°C

1.3.3 Enzyme CIAP

These are the graphs for the thermal stability of enzyme CIAP. The concentration of p-NP was calculated by using equation 2.

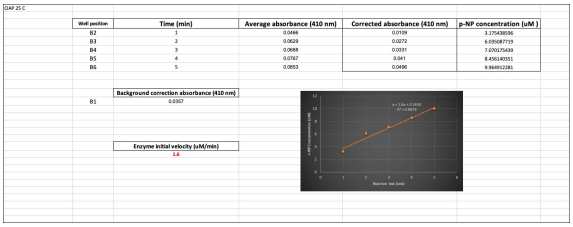


Figure 4: CIAP performance at 25°C

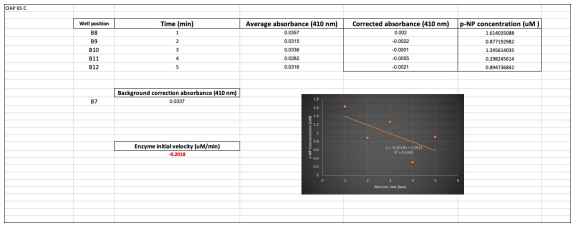


Figure 5: CIAP performance at 65°C

1.3.4 Relative Activity

The relative activity of enzymes BAP and CIAP according to the temperature are displayed in the following graphs.

1.4 Discussion

The BAP enzyme is activated when the temperature increases. Its relative activity is 1.26 at 65°C. This indicates that this enzyme gets more effective at 65°C rather than at 25°C.

On the other hand, the CIAP enzymes shows a negative results for its activity when the temperature is 65°C. This indicates that this enzyme gets deactivated when the temperature increases. Its relative activity is -0.126 at 65°C. Thus it can be inferred that the CIAP enzymes gets completely deactivated when the temperature increases. This is likely to happen due to

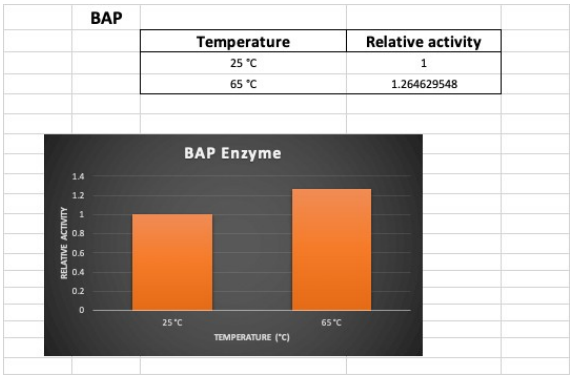


Figure 6: Relative activity of BAP enzyme

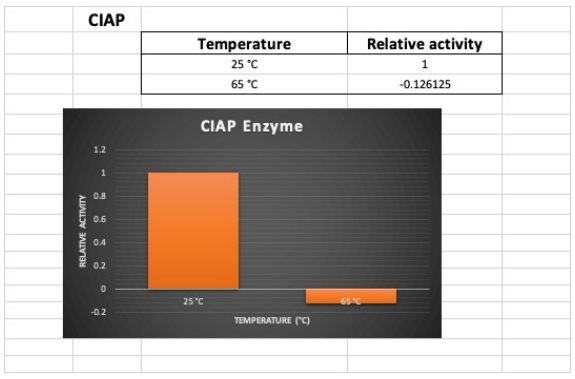


Figure 7: Relative activity of CIAP enzyme

its structural conformation.

Besides, the nature of the enzymes also determine the temperature that they can stand. For instance, BAP is found in E. coli, whereas CIAP is found in the small intestine of calf. This goes according to the results since E. coli can survive at higher temperatures compared to the small intestine of a calf.

1.5 Conclusion

It can be concluded that BAP enzyme is activated when the temperature increase. This is that BAP enzyme increases its activity when the temperature is 65°C compared to 25°C. On the other hand, the CIAP enzyme is completely deactivated when the temperature increases. The CIAP enzyme does not perform at 65°C.

SECOND EXPERIMENT

Conducted on November 17, Thursday

2 Inactivation of Alkaline Phosphatase: Impact of EDTA surfactant

2.1 Introduction

Enzymes are biocatalysts that are now widely used in various fields. One of the most basic and useful analytical methods for determining the catalytic properties of enzymes is kinetic analysis. By analyzing enzymatic reactions along a time axis, one can infer the reaction mechanism of newly isolated enzymes or enzymes in a specific reaction field. In this experiment, the Michaelis constant (Km) and maximum reaction rate (Vmax) were determined by first rate analysis using the hydrolysis reaction of phosphate ester by alkaline phosphatase (BAP) from Escherichia coli as a model. The same analysis will be conducted using alkaline phosphatase (CIAP) from small intestine of calf to study the differences in enzyme characteristics depending on the origin of the enzyme. In addition, we will deepen our understanding of enzyme inactivation and handling as a protein.
In Experiment 2, the effects of EDTA and surfactants will be investigated.

2.1.1 Materials

Reagents

- (a) Buffer solution: 10 mM Tris-HCI buffer (pH 8.0) 10 ml
- (b) Product solution: 0.10 mM p-nitroferol (p-NP) solution (pH 8.0) 1.0 ml
- (c) Substrate solution: 0.25 mM p-nitrophenyl phosphate (p-NPP) solution (pH 8.0) 6.0 ml
- (d) Enzyme solution 1: 0.10 mg/ml BAP solution (pH 8.0) 0.10 ml

- (e) 25 mM EDTA solution (10 mM Tris-HCI slack solution, pH 8.0) 1.0 ml
- (f) 2 wt% sodium dodecyl sulfate (SDS) solution (10mM Tris-HCI buffer, pH8.0) 1.0ml
- (g) Stopping solution: 1M sodium hydroxide solution 6.0 ml

Glasswares

- 96-well plate
- Eppendorf microtubes
- Two 10-100 ul adjustable micropipettes
- One 100-1000 ul adjustable micropipettes

Instruments

- Microplate Reader

2.2 Methodology

2.2.1 Preparation of product calibration curve

(1) 100 pl of Stopping Solution (g) were added to each well (C7-C12) of a 96-well plastic plate.
(2) 0.2 ml of aqueous product p-NP solution at various concentrations of [p-NP]=2.5, 5.0, 15, 25, 50 uM was prepared using buffer (a) and product solution (b).
(3) 100 ul of buffer solution (a) was added to the well of C7.
(4) 100 pl of the product solution of (2) was added to lanes C8 to C12.

2.2.2 Evaluation of enzyme reaction in the presence of EDTA

(1) 100ul of stopping solution (g) was added to each well ((D-F)×(1-6)) of a 96-well plastic plate.
(2) The plate was then incubated for at least 15 minutes.
(3) 0.5 ml of [EDTA]=2.5 mM EDTA solution (pH 8.0) was prepared using buffer solution (a) and 25 mM EDTA solution (e). 100 ul each of solution (e) (25 mM EDTA solution) and 2.5 mM EDTA aqueous solution prepared as described above were measured separately into

two Eppendorf tubes, to which 0.35 ml of buffer (a) was added. To each solution, 50 ul of enzyme solution (d) was added respectively, and allowed to stand for at least 10 minutes.
(4) Enzyme activity in the absence of EDTA was measured using the enzyme solutions in (2).
First, 100 ul of substrate solution (c) was added to the well of D1. Next, 0.90 ml of substrate solution (c) was added to a new microtube and 0.10 ml of the enzyme solution in (2) was added. The microtubes were quickly inverted to homogenize the solution and start the reaction. 100 ul of the reaction solution was added sequentially to each well of D2 to D6, sampling every 1 min for 5 min.
(5) The same procedure was performed for the enzyme solutions containing different concentrations of EDTA ([EDTA]=0.50 mM (lane E) and 5.0 mM (lane F)).

2.2.3 Evaluation of enzyme reaction in the presence of SDS

(1) To each well ((E,F)×(7-12)) of a 96-well plastic plate, 100 pl of stopping solution (g) was added.
(2) 0.5 ml of [SDS]=0.2 wt% SDS solution (pH 8.0) was prepared using buffer (a) and 2 wt% SDS solution (f). 0.25 ml each of 2 wt% SDS solution (f) and prepared 0.2 wt% SDS solution was measured separately into two microtubes, and added to these (a) was added to 0.20 ml of each solution. To each solution, 50 ul of enzyme solution (d) was added and allowed to stand for at least 10 minutes.
(3) Enzyme activity was measured using the enzyme solutions in (2).
First, 100 ul of substrate solution (c) was added to the wells of E7. Next, 0.90 ml of substrate solution (c) was added to a new microtube, 0.10 ml of the enzyme solution in 0.1 wt% SDS of was added, and the microtube was quickly inverted to homogenize the solution and start the reaction. 100 ul of the reaction solution was sampled every 1 min for 5 min and added sequentially to each well of E8 to E12.
(4) The operation described in (3) was performed in the same way for enzyme solutions containing different concentrations of SDS ([SDS]=1.0 wt% (F lane)) prepared in (2).

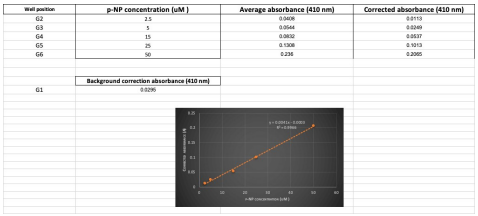
The absorbance at 410 nm of each well in experiments 2.2.1, 2.2.2, and 2.2.3 was measured by using a microplate reader.

2.3 Results

The results of the experiment were analyzed and are displayed as follows.

2.3.1 Background calibration

The background calibration curve was analyzed using solution (b) Product solution: 0.10 mM p-nitroferol (p-NP).



Well position	p-NP concentration (uM)	Average absorbance (410 nm)	Corrected absorbance (410 nm)
G2	2.5	0.0408	0.0113
G3	5	0.0544	0.0249
G4	15	0.0832	0.0537
G5	25	0.1308	0.1013
G6	50	0.236	0.2065

Background correction absorbance (410 nm)
G1	0.0295

Figure 8: Background calibration

Here, the calibration is conducted according to the linear equation 3.

$$y = ax - b \tag{3}$$

In which x represents the concentration of p-NP, and y represents the corrected absorbance. The values of a and b constants are as follows:

$$a = 0.0041$$

$$b = 0.0003$$

Therefore, the concentration of p-NP can be calculated from the corrected absorbance measured by using equation 4.

$$x = \frac{y + b}{a} \tag{4}$$

2.3.2 Reaction with presence of EDTA

These are the graphs for the reaction in presence of EDTA. The concentration of p-NP was calculated by using equation 6.

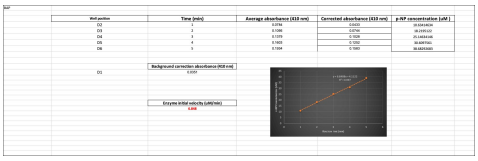


Figure 9: No presence of EDTA (positive control)

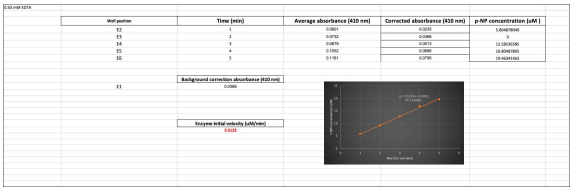


Figure 10: 0.5 mM EDTA

Figure 11: 5.0 mM EDTA

2.3.3 Reaction with presence of SDS

These are the graphs for the reactions in presence of SDS. The concentration of p-NP was calculated by using equation 6.

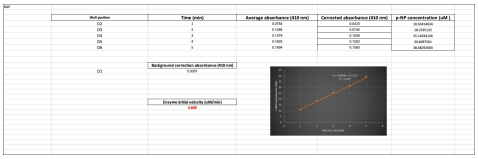


Figure 12: No presence of SDS (positive control)

Figure 13: 0.1 wt% of SDS

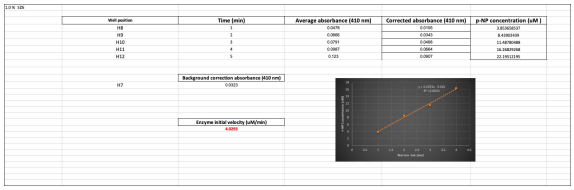


Figure 14: 1.0 wt% of SDS

2.3.4 Relative Activity

The relative activity of EDTA and SDS according to their concentration in the reaction are displayed in the following graphs.

2.4 Discussion

The results show that EDTA decreases the activity of enzyme BAP. Whereas the SDS also decreases it, but in a more subtle way.

When EDTA is present in a 5.0 mM concentration, the BAP enzyme is not able to perform. Thus the EDTA is a strong deactivator of this enzyme.

On the other hand, even when SDS is present in 1.0 wt% concentration, the deactivation of the BAP enzyme is not as strong as it is when EDTA is present.

EDTA

Concentration	Relative activity
BAP (positive control)	1
0.50 mM EDTA	0.512879673
5.0 mM EDTA	-0.002847547

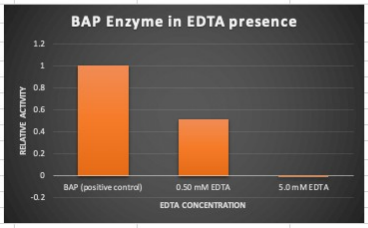


Figure 15: Relative activity of EDTA

SDS

Concentration	Relative activity
BAP (positive control)	1
0.1 wt% SDS	0.814544393
1.0 wt% SDS	0.588390771

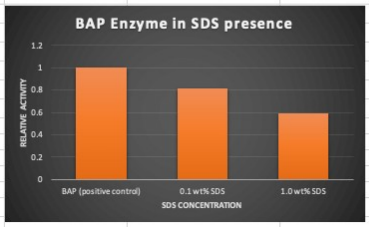


Figure 16: Relative activity of SDS

2.5 Conclusion

It can be concluded that EDTA strongly deactivates the action of enzyme BAP. On the other hand, SDS is also an inhibitor of the BAP enzyme, however it is not as strong as the effect caused by the presence of EDTA.

THIRD EXPERIMENT

Conducted on November 30, Wednesday

3 Kinetic analysis of BAP and CIAP catalyzed reactions

3.1 Introduction

Enzymes are biocatalysts that are now widely used in various fields. One of the most basic and useful analytical methods for determining the catalytic properties of enzymes is kinetic analysis. By analyzing enzymatic reactions along a time axis, one can infer the reaction mechanism of newly isolated enzymes or enzymes in a specific reaction field. In this experiment, the Michaelis constant (Km) and maximum reaction rate (Vmax) were determined by first rate analysis using the hydrolysis reaction of phosphate ester by alkaline phosphatase (BAP) from Escherichia coli as a model. The same analysis will be conducted using alkaline phosphatase (CIAP) from small intestine of calf to study the differences in enzyme characteristics depending on the origin of the enzyme. In addition, we will deepen our understanding of enzyme inactivation and handling as a protein. In Experiment 3, the kinetics of p-nitrophenyl phosphate hydrolysis by BAP and CIAP will be analyzed.

3.1.1 Materials

Reagents

- (a) Buffer solution: 10 mM Tris-HCI buffer (pH 8.0) 10 ml
- (b) Product solution: 0.10 mM p-nitroferol (p-NP) solution (pH 8.0) 1.0 ml
- (c) Substrate solution: 0.25 mM p-nitrophenyl phosphate (p-NPP) solution (pH 8.0) 5.0 ml
- (d) Enzyme solution 1: 0.10 mg/ml BAP solution (pH 8.0) 0.10 ml
- (e) Enzyme solution 2: 5.4 ug/ml CIAP solution (pH 8.0) 0.10 ml

- (f) Stopping solution: 1M sodium hydroxide solution 10 ml

Glasswares

- 96-well plate
- Eppendorf microtubes
- Two 10-100 ul adjustable micropipettes
- One 100-1000 ul adjustable micropipettes

Instruments

- Microplate Reader

3.2 Methodology

3.2.1 Preparation of product calibration curve

(1) 100 pl of Stopping Solution (f) were added to each well (G1-G6) of a 96-well plastic plate.
(2) 0.2 ml of aqueous product p-NP solution at various concentrations of [p-NP]=2.5, 5.0, 15, 25, 50 uM was prepared using buffer (a) and product solution (b).
(3) 100 ul of buffer solution (a) was added to the well of G1.
(4) 100 pl of the product solution of (2) was added to lanes G2 to G6.

3.2.2 Evaluation of of BAP enzyme reaction

(1) Add 100 ul of stopping solution (f) to each well ((A-E)×(1-6)) of a 96-well plastic plate.
(2) Buffer (a) and 1.0 mM p-NPP solution (C) were used to prepare 1.5 ml (1.4 ml only for 7.5 uM) of [p-NPP]=7.5, 10, 15, 25, 50 uM p-NPP solution.
(3) 100 ul of the [p-NPP]=7.5 uM substrate solution prepared in (2) was added to the well of A1.
(4) In a new microtube, 1.0 ml of the substrate solution of [p-NPP]=7.5 uM prepared in (2) was measured. 10 ul of enzyme solution (d) (BAP) was added to 1.0 ml of the measured substrate solution and the microtubes were quickly inverted to homogenize the solution and start the reaction. 100 ul of the reaction solution was sampled every minute for 5 minutes

and added sequentially to each well from A2 to A6.

(5) The solutions preparede in (3) and (4) were performed in the same manner for each substrate solution of [p-NPP]= 10 (Lane B), 15 (Lane C), 25 (Lane D), and 50 (Lane E) uM.

3.2.3 Evaluation of CIAP enzyme reaction

(1) To each well ((A-E)×(7-12)) of a 96-well plastic plate, 100 ul of stopping solution (f) was added.

(2) 1.5 ml of substrate solution at concentrations of [p-NPP]=0.10, 0.25, 0.35, 0.50, 0.75 mM was prepared using buffer solution (a) and 1.0 mM p-NPP solution (c).

(3) 100 ul of the [p-NPP] = 0.10 mM substrate solution prepared in (2) was added to the wells of A7.

(4) In a new microtube, 1.0 ml of the [p-NPP] = 0.10 mM substrate solution prepared in (2) was measured. 10 ul of enzyme solution (e) (CIAP) was added to 1.0 ml of the measured substrate solution and the quick Eppendorf tube was inverted to homogenize the solution and start the reaction. 100 ul of the reaction solution was added sequentially to A8 to A12, sampling every 1 min for 5 min.

(5) The solutions prepared in (3) and (4) were analyzed in the same manner for each substrate solution with [p-NPP] = 0.25 (lane B), 0.35 (lane C), 0.50 (lane D), and 0.75 (lane E) mM.

The absorbance at 410 nm of each well in experiments 3.2.1, 3.2.2, and 3.2.3 was measured by using a microplate reader.

3.3 Results

The results of the experiment were analyzed and are displayed as follows.

3.3.1 Background calibration

The background calibration curve was analyzed using solution (b) Product solution: 0.10 mM p-nitroferol (p-NP).

Here, the calibration is conducted according to the linear equation 5.

$$y = ax - b \tag{5}$$

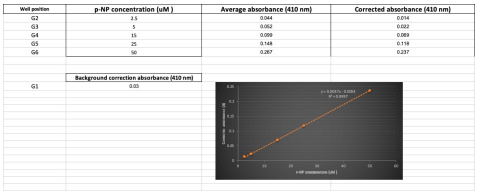


Figure 17: Background calibration

In which x represents the concentration of p-NP, and y represents the corrected absorbance. The values of a and b constants are as follows:

$$a = 0.0047$$

$$b = 0.0004$$

Therefore, the concentration of p-NP can be calculated from the corrected absorbance measured by using equation 6.

$$x = \frac{y + b}{a} \tag{6}$$

3.3.2 Reaction with enzyme BAP

These are the graphs for the reaction of p-NPP with the enzyme BAP. The concentration of p-NPP is adjusted in different repitions in order to study the kinetics of the BAP enzyme. The concentration of p-NP was calculated by using equation 4.

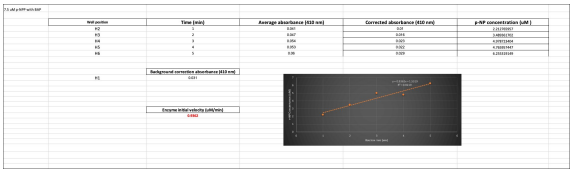


Figure 18: 7.5 uM p-NPP

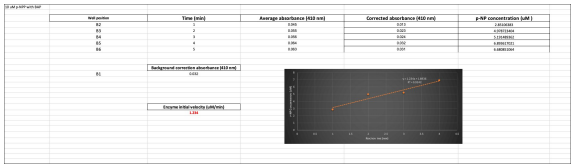


Figure 19: 10 uM p-NPP

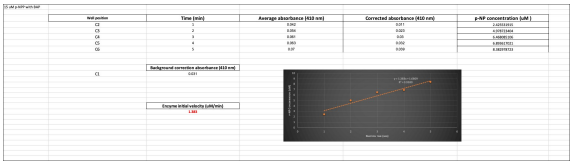


Figure 20: 15 uM p-NPP

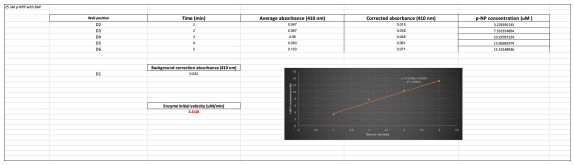


Figure 21: 25 uM p-NPP

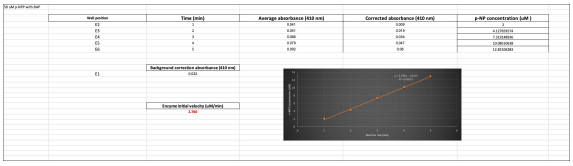


Figure 22: 50 uM p-NPP

3.3.3 Reaction with enzyme CIAP

These are the graphs for the reaction of p-NPP with the enzyme CIAP. The concentration of p-NPP is adjusted in different repitions in order to study the kinetics of the CIAP enzyme. The concentration of p-NP was calculated by using equation 4.

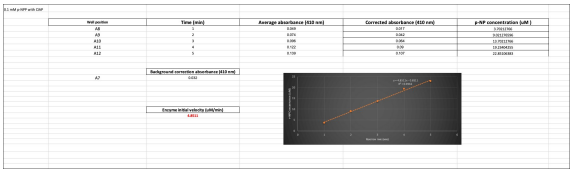


Figure 23: 100 uM p-NPP

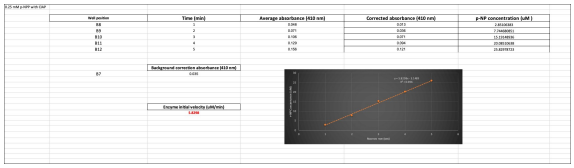


Figure 24: 250 uM p-NPP

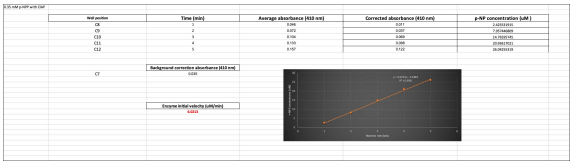


Figure 25: 350 uM p-NPP

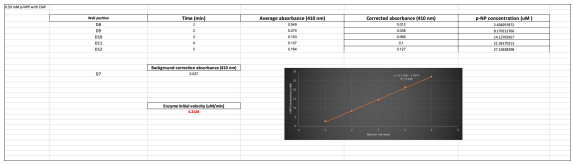


Figure 26: 500 uM p-NPP

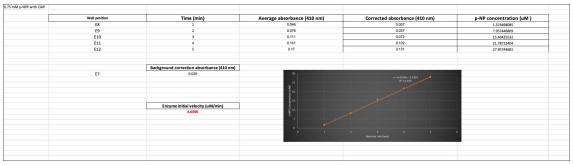


Figure 27: 750 uM p-NPP

3.3.4 Applying the Michaelis-Menten equation

The Michaelis-Menten equation was applied for the reactions with enzyme BAP and enzyme CIAP. The graphs are shown below.

Michaelis-Menten (BAP)

p-NPP Concentration (uM)	Enzyme initial velocity Vo (uM/min)
7.5	0.9362
10	1.234
15	1.383
25	3.2128
50	2.766

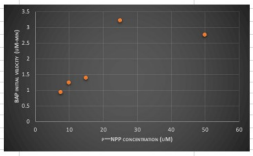


Figure 28: Michaelis-Menten graph for BAP

Michaelis-Menten (CIAP)

p-NPP Concentration (uM)	Enzyme initial velocity Vo (uM/min)
100	4.8511
250	5.8298
350	6.0213
500	6.2128
750	6.6596

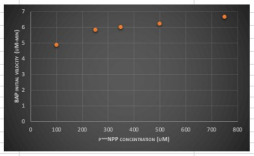


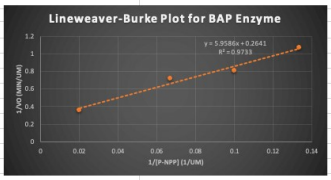
Figure 29: Michaelis-Menten graph for CIAP

3.3.5 Applying the Lineweaver-Burke Plot

The Lineweaver-Burke Plot was applied for the Michaelis-Menten equation of reactions with enzyme BAP and enzyme CIAP. The graphs are shown below.

Lineweaver-Burke Plot (BAP)

1/[p-NPP] (1/uM)	1/Vo (min/uM)
0.133333333	1.068147832
0.1	0.810372771
0.066666667	0.723065799
0.04	0.31125498
0.02	0.361532899



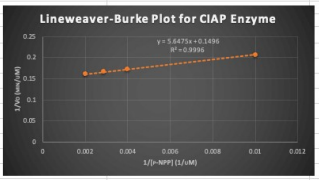
1/Vmax (min/uM):	0.2641
Vmax (uM/min):	**3.786444529**
Vmax (umol/min mg):	**3.786444529**

Km/Vmax (min):	5.9586
Km (uM):	**22.56190837**

Figure 30: Lineweaver-Burke plot for BAP

Lineweaver-Burke Plot (CIAP)

1/[p-NPP] (1/uM)	1/Vo (min/uM)
0.01	0.206138814
0.004	0.171532471
0.002857143	0.166077093
0.002	0.160958022
0.001333333	0.150159169



1/Vmax (min/uM):	0.1496
Vmax (uM/min):	**6.684491979**
Vmax (umol/min mg):	**123.7868885**

Km/Vmax (min):	5.6475
Km (uM):	**37.75066845**

Figure 31: Lineweaver-Burke plot for CIAP

3.4 Discussion

The graphs are according to expected. The deactivation of BAP and CIAP enzymes could be studied in this experiment.

BAP results:

$$Vmax = 3.8\mu\text{mol}\,\text{min}^{-1}\,\text{mg}^{-1}$$

$$Km = 23\mu\text{M}$$

CIAP results:

$$Vmax = 1.2 * 10^{2}\mu\text{mol}\,\text{min}^{-1}\,\text{mg}^{-1}$$

$$Km = 38\mu\text{M}$$

The Km represents the Michaelis constant, and the Vmax represents the maximum reaction rate for each enzyme.

3.5 Conclusion

It can be concluded that enzyme BAP has a slower action than enzyme CIAP. This is because the maximum velocity is significantly smaller than that of CIAP. Besides, since the Km value is smaller for the BAP, the p-NPP solution has a higher affinity for the enzyme BAP than it has for the enzyme CIAP.

4 Assignment

The answers to the questions of the assignment are explained here.

1. Alkaline phosphatase (ALP) is an enzyme used in the diagnosis of bone disease, hepatobiliary disease, and cancer. ALP is also an enzyme whose catalytic reaction can be applied to various experiments in the life sciences. It is also an enzyme whose catalytic reaction can be applied to various experiments in the life sciences. By definition, alkaline phosphatase is an enzyme with low substrate specificity that hydrolyzes phosphate monoester bonds in alkaline conditions (pH 9-11).

2. BAP has a molecular weight of about 80,000 and an isoelectric point of 4.5. In contrast, CIAP has a molecular weight of approximately 100,000 and an isoelectric point of 5.7. BAP is an extremely stable enzyme, and CIAP is less stable than BAP. The kinetic constant Km is the substrate concentration when v is 1/2 of Vmax, based on the Michaelis-Menten equation, and represents the dissociation constant of the ES complex, indicating the affinity between E (enzyme molecules) and S (substrate molecules). The lower the Km value, the higher the affinity and the lower the concentration of substrate, the more likely the complex is to form. In other words, we can consider that the BAP with higher thermal stability has a lower kinetic constant because of its higher affinity, and the CIAP with lower thermal stability than the BAP has a higher kinetic constant because of its lower affinity.

3. Alkaline phosphatase is a zinc-containing dimeric enzyme, each subunit containing 429 amino acids with four cysteine residues linking the two subunits. Alkaline phosphatase contains four Zn ions and two Mg ions, with Zn occupying active sites A and B, and Mg occupying site C. The mechanism of action of alkaline phosphatase involves the geometric coordination of the substrate between the Zn ions in the active sites. The mechanism by which alkaline phosphatase hydrolyzes its substrate (phosphate ester) is shown below.

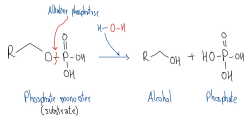


Figure 32: Mechanism of action of alkaline phosphatase

4. Deactivation, or inactivation, of an enzyme occurs when the enzyme loses its activity, and is no longer able to function as a catalyst. In the present experiment, deactivation was measured and quantitated by measuring the rate of the enzyme kinetics under specific conditions. According to the results, deactivation occured due to structural changes in the enzyme. In this case, it is likely to be due to aggregation (the enzyme proteins clumping together), causing the enzyme structure to unfold and lose integrity.

Enzyme inhibition is a common feature of metabolic pathway control in cells. Metabolic flux through a pathway is often regulated by a pathway's metabolites acting as inhibitors and enhancers for the enzymes in that same pathway. Therefore, another example of enzyme deactivation is the glycolytic pathway. This catabolic pathway consumes glucose and produces ATP, NADH and pyruvate. A key step for the regulation of glycolysis is an early reaction in the pathway catalysed by phosphofructokinase-1 (PFK1). When ATP levels rise, ATP binds an allosteric site in PFK1 to decrease the rate of the enzyme reaction; glycolysis is inhibited and ATP production falls. This negative feedback control helps maintain a steady concentration of ATP in the cell.

5. The important types of inhibitors are competitive, noncompetitive, and uncompetitive inhibitors. Competitive enzyme inhibitors possess a similar shape to that of the substrate molecule and compete with the substrate for the active site of the enzyme. This prevents the formation of enzyme-substrate complexes. Therefore, fewer substrate molecules can bind to the enzymes so the reaction rate is decreased. The level of inhibition depends on the relative concentration of substrate and inhibitor. This is a reversible process (temporary binding). In the case of competitive inhibition, Km is increased but Vmax is not altered. Noncompetitive enzyme inhibitors bind to a site other than the active site of the enzyme, called an allosteric site. Due to this binding, it deforms the structure of the enzyme so that it does not form the ES complex at its normal rate, and it prevents the formation of enzyme-product complexes, which leads to fewer product formations. Because they do not compete with substrate molecules, noncompetitive inhibitors are not affected by substrate concentration. In the case of noncompetitive inhibition, Vmax is lowered but Km is not altered. Uncompetitive inhibitor cannot bind to the free enzyme, but only to the ES complex. The resulting ES complex is enzymatically inactive. This type of inhibition is rare but may occur in multimeric enzymes. Some enzyme inhibitors can be used as a medicine or as metabolic poison in the treatment of a particular disease.

References

[1] Kuddus, M.. Enzymes in Food Biotechnology, 2019.

[2] Kathleen M.. The mechanism of the alkaline phosphatase reaction: insights from NMR, crystallography and site-specific mutagenesis, 1999.

[3] J. Ge. Comprehensive Biotechnology (Second Edition), 2011.