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Functional Analysis Of Bacillus Anthracis Aspartate Transcarbamoylase And Dihydroorotase

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FUNCTIONAL ANALYSIS OF *Bacillus anthracis* ASPARTATE TRANSCARBAMOYLASE AND DIHYDROOROTASE.

by

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THESIS

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DEDICATION

To Evelyn who gave me endless support through all my endeavors.

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Special thanks to Wayne State University School of Medicine Department of Biochemistry, Microbiology, and Immunology. Special thanks to my advisor Dr. David Evans as well as Chandni Patel and Lauren Iacobelli. Also, special thanks to my committee members Dr. Brian Edwards and Dr. Ladislav Kovari. I would also like to thank everyone who has supported me through my degree. Especially, my mother, Evelyn, who has given me unconditional support.

PREFACE

This thesis includes research undertaken to gain a clearer understanding of the effects of the *Bacillus anthracis* enzymes ATCase and DHOase in pyrimidine biosynthesis. The biochemical and structural aspects given by these enzymes is important to human health and will hopefully lead to a better understanding of the treatment of diseases such as bacteremia and anthrax.

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CHAPTER 1: INTRODUCTION

Anthrax

Anthrax is an infection caused by *Bacillus anthracis* (BCN). There are four types of anthrax infection: skin, lungs, intestinal, and injection. The most common form is skin anthrax. The most fatal is lung anthrax. Anthrax is typically characterized by a swollen blister or ulcer with a black center. This is often accompanied by fever, chest pain, shortness of breath, abscesses, diarrhea, nausea, and/or vomiting depending on the type of anthrax exhibited. This disease is not spread through direct human to human interaction, but rather through the exposure to bacterial spores. Anthrax is a relatively rare disease, but it is seen most frequently in Africa and parts of Asia. A vaccine exists for anthrax. This serious zoonotic disease is mostly associated with animals.[1] However, anthrax also infects humans. Anthrax infection is expressed in four different forms. The four forms are skin lesions or cutaneous anthrax, lung or pulmonary anthrax, gastrointestinal anthrax, and anthrax from injections. The most common form of anthrax is cutaneous anthrax of the skin. Cutaneous anthrax is also considered to be the least dangerous. Cutaneous anthrax typically develops within 1 to 7 days. The most dangerous form of anthrax is pulmonary anthrax of the lung. It can take up to two months to fully develop. Gastrointestinal anthrax is relatively rare but takes anywhere from 1 to 7 days to develop. Injection anthrax has never been reported in the United States. The disease is spread when *Bacillus anthracis* spores enter the body. [2] Bacillus anthracis is a gram positive, rod shaped bacteria. There has been research into the use of anthrax as a biochemical weapon and has been seen in bioterrorism. [3] Lung anthrax is especially used in biowarfare. [4] It can be delivered as an aerosol and spread through the air.

Therefore, anthrax has been a hot topic of research interest. If anthrax is left untreated, it is usually fatal. [5] In addition to anthrax, bacteremia is another disease caused by Bacillus *anthracis*. [6] This is of high importance in the hospital setting, as it is a cause of mortality in medical facilities.

Bacteremia

Bacteremia, also called septicemia, is the presence of bacteria in the bloodstream. When bacteria enter the bloodstream, it results in systemic inflammation and septic shock leading to death. It develops when bacteria evade the immune response mechanisms in the body. [7] Specifically, when certain bacteria, such as Bacillus anthracis, get into the bloodstream, the growth is exponential. Bacteremia can start in multiple ways from a simple cut to medical procedures. Therefore, bacteremia is most common in a medical facility. Bacteremia is commonly confused with sepsis. Sepsis occurs when bacteria enter the body and causes inflammation and coagulation. [8] Specifically, it causes the release of proinflammatory cytokines, procoagulants, and adhesion molecules. Sepsis can lead to a precipitous decrease in blood pressure, called septic shock, which is often fatal. It is characterized by fever, and heavy breathing. Whereas bacteremia can vary in urgency, sepsis is almost always severe. Currently, antibiotics can be used to treat sepsis and bacteremia, but no cure exists. With the rise in antibiotic resistant bacteria, new and improved antibiotics and preventative measures must be made.

Pyrimidine Biosynthesis

Nucleoside antibiotics are products that block or destroy the complex regulatory mechanisms necessary for bacterial survival in a host. [9] Pyrimidine biosynthesis is essential to all living organisms, including bacteria. Pyrimidine

biosynthesis could potentially be inhibited by an antibiotic, thus killing the bacteria that was causing infection. The production of such an inhibitor may ultimately lead to the development of new treatments for bacteremia and sepsis. There are many enzymes required for efficient and proper pyrimidine biosynthesis. Two that are very important and will be discussed in this thesis is aspartate transcarbamoylase (ATCase) and dihydroorotase (DHOase). ATCase plays an important role in not only pyrimidine biosynthesis production, but also in the mechanistic regulation of de novo synthesis. DHOase, however, is unregulated. Pyrimidines are nitrogen containing heterocyclic compounds with basic properties. [10] The nucleobases, cytosine, thymine, and uracil are nucleic acid derivatives of pyrimidines. These are the “building blocks of life” and are needed for metabolic functions, such as DNA replication and formation. In order for bacteria to survive in the blood stream, basic metabolic functions must be conserved. Bacteria can utilize some nutrients in the host’s bloodstream, but other nutrients need to be produced *de novo*. The production of pyrimidines is one such mechanism that is critical for bacterial growth in blood. [11] Pyrimidines are not readily available in blood, so bacteria must rely on their own production of pyrimidines to survive in a host environment There are two ways to make pyrimidines. One is through the salvage pathway, and the other is *de novo*. However, the precursors for the salvage pathway are too low in the blood stream. Therefore, bacteria rely solely on *de novo* synthesis. ATCase and DHOase are important enzymes in the biosynthesis of pyrimidines in bacteria and consequentially can be a drug design target. [12] Thus, the creation of an antibiotic that targets ATCase or DHOase could cure anthrax disease or bacteremia caused by *Bacillus anthracis*. Previous studies have also showed that a complex between ATCase and DHOase forms

in other bacterial species, such as *Aquifex aeolicus*. [13] Other studies show that in some microorganisms there is an active DHOase and in others there is a pseudo DHOase which retains the structure but has no activity. [14] However, the DHOase in *Bacillus anthracis* is indeed active.

Aspartate Transcarbamoylase

Aspartate transcarbamyolase is the enzyme protein that catalyzes the first step in production of pyrimidines. It condenses L-aspartate and carbamoyl phosphate into N-carbamy-L-aspartate and inorganic phosphate. In *E.coli* the following statements about ATCase regulation are true. [15] Cytosine triphosphate and uridine triphosphate (CTP and UTP) are products of pyrimidine synthesis. CTP decreases activity of ATCase. Adenine triphosphate (ATP) is a product of purine synthesis (a similar yet opposite synthetic mechanism). ATP increases the activity of ATCase. Thus, ATCase's allosteric regulation is seen. In *E. coli,* it consists of two trimeric catalytic subunits and 3 dimeric regulatory subunits. [16] Yet, this structural composition varies between species. [17] Some are just catalytic trimers, not associated with any function. Some have regulatory subunits, others like the ones I have studied in this thesis have an active DHOase or an inactive pseudo DHOase. Others associate with carbamoyl phosphate synthetase. Mammalian ATCase is a part of CAD. CAD is a 240-kDa polypeptide with many functions. [18] In *Pseudomonas aeruginosa*, active ATCase contains six copies of a 36-kDa catalytic chains and six copies of a 45-kDa pseudo DHOase. [19] In *E. coli,* ATCase contains a catalytic center and an allosteric site. The protein structure for ATCase in BCN has yet to be determined.

Dihydroorotase

Dihydroorotase (DHOase) is a member of the MEROPS peptidase family M38 (clan MJ). DHOase is a dimeric molecule in most organisms. It also has identical chains of approximately 400 amino acids. [20] DHOase catalyzes the third step in the de novo biosynthesis of pyrimidines. [21] It converts ureidosuccinic acid (N-carbamoyl-L-aspartate) into dihydroorotate and requires one or two zinc atoms for catalytic activity in all species. [22]

CHAPTER 2: RESEARCH DESIGN

Materials

The reagents used in this project were sourced as follows: **B-D Chemicals**: yeast extract, tryptones; **BioRad**: Tris Chloride, Acrylamide; **Fisher**: HCL, Bovine serum albumin; **Gold Bio**: Ampicillin, IPTG, DTT; **Invitrogen**: Competent BL21 E. Coli cells, SDS, TEM-Ed; **Lucigen**: Recovery media; **Mallinckrodt**: Nickel (II) chloride, Sodium carbonate; **Novex**: Nickel affinity column; **Roche**: DNAse; **RPI**: Terrific broth media; **Sigma-Aldrich**: Sodium chloride, Glycerol, Folin reagent; **Sigma**: ATP, GTP, CTP, and TTP, L-aspartate, Sodium acetate, Antipyrine, Diacetyl monooxime, Dihydroorotate, Sodium phosphate, Sodium hydroxide, Imidazole, Brilliant blue stain, Carbamoyl aspartate, Carbamoyl phosphate; **Thermo-Fisher**: bis(sulfosuccinimidyl)suberate (BS3), Sypro.

Protein Expression

Prior to all experiments, the ATCase and DHOase protein sequence of *Bacillus anthracis* was aligned with multiple other species to see homologies among species. Our

species retained all important active sites conserved among the other species. Bacterial expression of the proteins were performed. *Bacillus anthracis* ATCase and DHOase plasmids were transfected into BL21(DE3) *E. coli* cells. Cells were then grown to an optical density of 0.6 at 700 nm wavelength and harvested by centrifugation at 2000 RPM for 50 minutes. The bacteria plasmid was then added to 20ul of BL21(DE3) competent cells in 1 ml recovery media. The reactions were incubated at 37°C and plated on LB ampicillin plates. The plates were then incubated at 37°C overnight.

Cell Growth and Harvesting

Single colonies were inoculated into 10 mL TB media. Stocks were then saved at -80°C in 25% glycerol. All cells were grown to an optical density (OD) of approximately 0.6 absorbance units at 600nm wavelength. They were then induced with 1mM IPTG overnight at 20°C. The cells were harvested using centrifugation at 2000 rpm for 50 minutes.

Protein Purification

The enzyme was purified using Ni-column chromatography and gel filtration. Ni-columns have the ability to bind His6 tagged proteins to the nickel in the column. The column was washed with purification buffer followed by increasing Imidazole solutions to elute the tagged protein. Imidazole competes for binding with the protein to the nickel column. Gel filtration, or size exclusion chromatography was done to remove any contaminants. A S200 column of approximately 120 ml was used to purify the large proteins. The protein concentration was checked with the Lowry assay, using bovine serum albumin as the standard. The molecular mass of the recombinant proteins was determined by gel filtration on an AKTA chromatography system. A Ni^{2+} column can

purify his-tagged DHOase and ATCase. A Superdex S-200 column was calibrated with 50 mM Tris-HCl, pH 7.5, 200 mM NaCl, and 1 mM dithiothreitol. A sample of purified protein was applied and eluted with the same buffer at a flow rate of 1 mL/min.

CHAPTER 3: ASSAYS

Lowry Assay

A Lowry assay was used to determine the concentration of each enzyme after expression and purification. These concentrations were determined by using bovine serum albumin 1mg/ml as a standard.

Calorimetric ATCase and DHOase Activity Assays

Assays were then performed of the enzymes. Activity assays such as the ATCase calorimetric assay was performed. The assay was performed using 0.01M carbamoyl aspartate, 48mM carbamoyl phosphate, 0.3M L-aspartate, 0.1M Tris-OAc at pH 8.3, antipyrine reagent, and color mix. The reactions were incubated at 37°C for exactly two minutes and then quenched with 5% acetic acid. The reaction mixtures were then incubated at 60°C for one hour. The activity was measured by checking the absorbance at 466nm using a DU70 spectrophotometer.

A DHOase colorimetric assay was also performed. The assay was performed using 5mM carbamoyl aspartate, 8mM Dihydroorotate, 0.1M Tris-OAc at pH 8.3, antipyrine reagent, and antipyrine/diacetyl monooxime color mix. The reactions were incubated at 37°C for exactly two minutes and then quenched with 5% acetic acid. The reaction mixtures were then incubated at 60°C for one hour. The activity was measured using a DU70 spectrophotometer.

Crosslinking

The crosslinking assay was used to isolate and characterize the two enzymes in a protein protein interaction. This assay was able to freeze and capture the two proteins bound to each other. 50 nM protein concentrations were added to 1mM BS3. 10 ul aliquots were then taken from the reaction after 0, 5, 10, 30 60, and 120-minute intervals and quenched with 10ml 1M Tris, pH 8. Characterization was then shown using sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Thermal Shift Assay

A thermal shift assay was done to detect the enzymes' thermal stability. It determined the protein's melting temperature to find its thermal stability. It was also able to identify intramolecular and intermolecular interactions. The assay was done in a 96-well PCR microplate from Applied Biosystems. Each well contained 1 ul of purified protein, 1 ul 500X SYPRO Orange dye (5000X diluted into buffer), and 23 ul buffer (50mM sodium phosphate pH 7.5, 500mM NaCl, and 5% glycerol). An adhesive plastic cover was applied to the plate and was centrifuged manually 5 times. The melting temperatures of the enzymes were calculated by the first derivative of the fluorescence vs. temperature plot using the Applied Biosystems 7500 software.

Nucleotide Inhibition Assay

An activity assay was used to screen inhibitors such as nucleotides, which act on these enzymes. This was done using the colorimetric assay. Enzyme kinetics was performed to determine the enzyme's activity. Inhibition was determined to measure the affinity of the enzyme. Steady-state kinetics was performed using different inhibitors to determine whether the inhibitor is competitive or noncompetitive. The ATCase assay was

performed using 2mM aspartate, 50mM Tris/Acetate buffer of pH 8.3, 2-3 μg purified ATC in 1 ml volume, 2 ml color mix (antipyrine/diacetyl monooxime) and was incubated at 60°C for one hour. The reaction was initiated with 0-5 mM carbamoyl phosphate for 2 minutes at 37°C. DHOase activity was initiated with 0-8mM DHO for 2 minutes at 37°C. The assay was performed with 50mM Tris acetate buffer at pH 8.3, 25 μg purified DHO in 1 ml volume, and 2 ml color mix.

CHAPTER 4: RESULTS

Protein Purification and Expression

Bacillus anthracis ATCase and DHOase were expressed and purified as shown in Figure 1 (ATCase) and Figure 2 (DHOase).

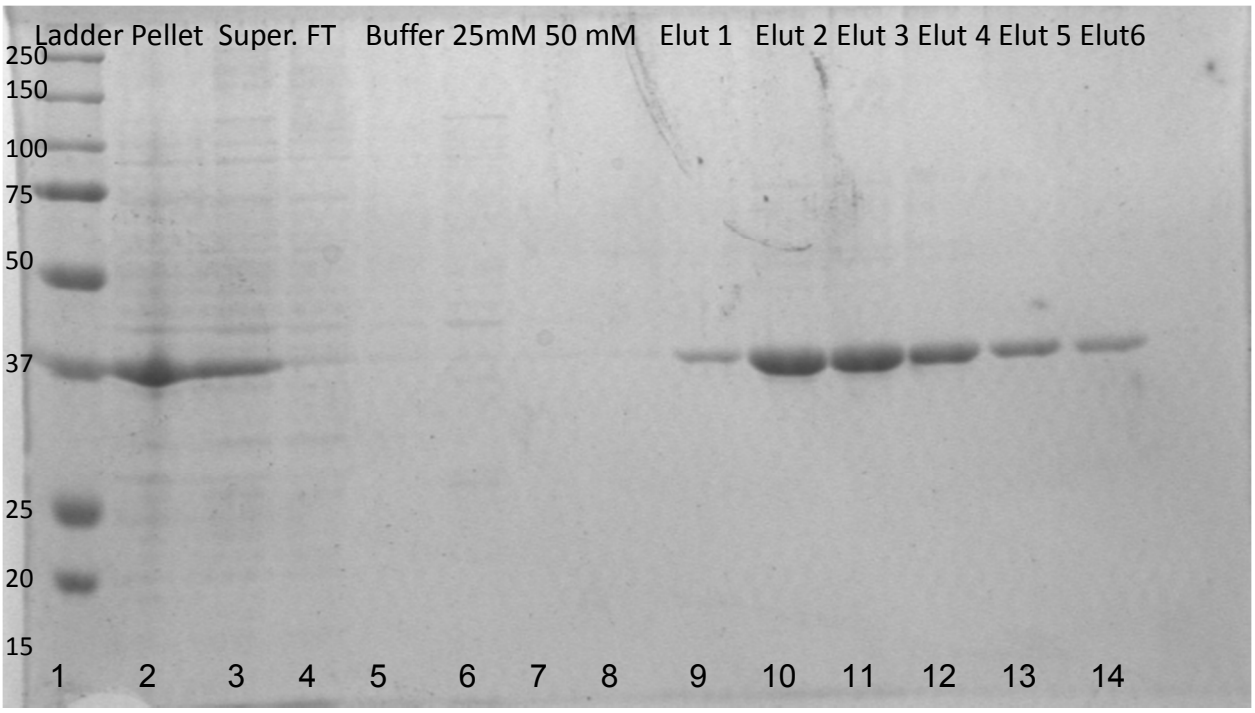

Figure 1. Expression and purification of ATCase. Ladder, pellet, super (supernatant), FT (flow through). The protein was then eluted with 0mM Buffer, 25mM imidazole, 50 mM

imidazole, and 250mM imidiazole (elute 1, elute 2, elute 3, elute 4, elute 5 and elute 6) of 4 ml elutions each.

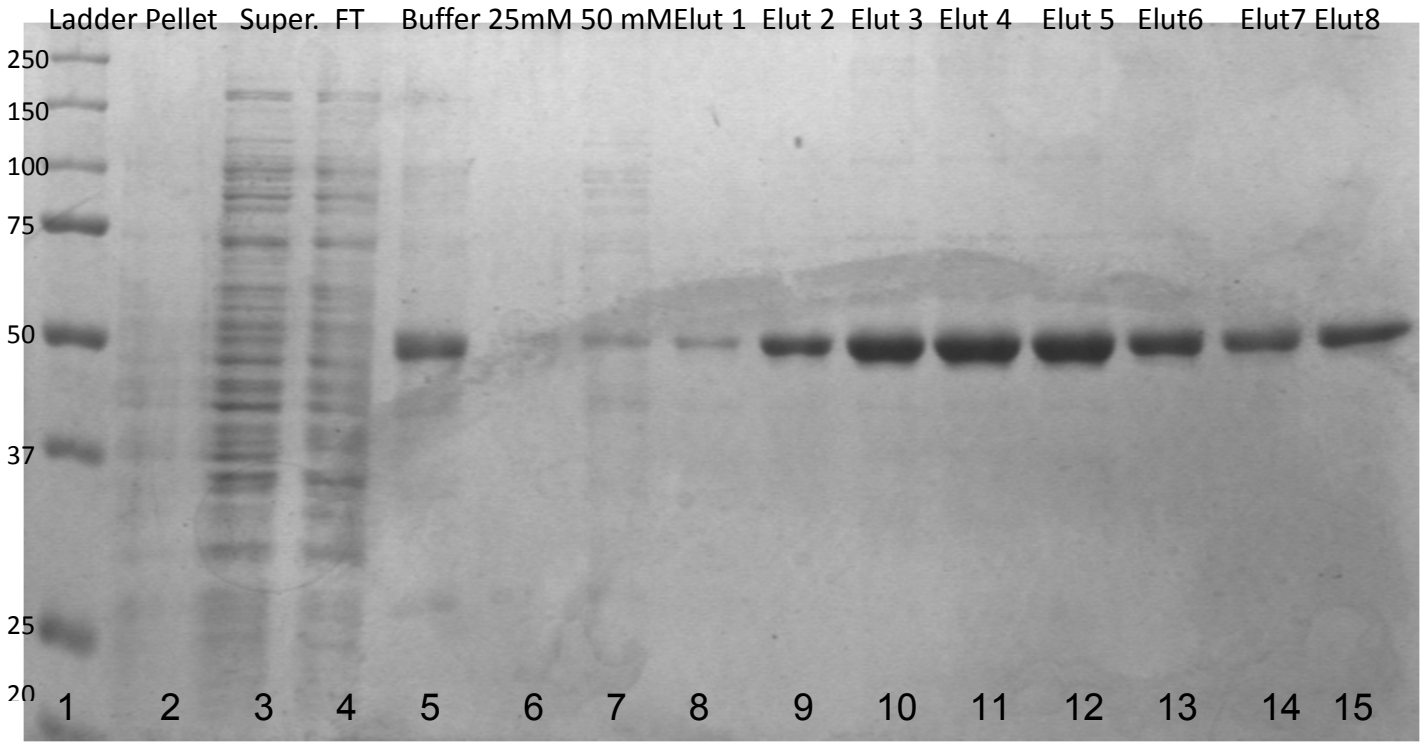

Figure 2. Expression and purification of DHOase. Ladder, pellet, super (supernatant), FT (flow through). The protein was then eluted with 0mM Buffer, 25mM imidazole, 50 mM imidazole, and 250mM imidiazole (elute 1, elute 2, elute 3, elute 4, elute 5, elute 6, elute 7, and elute 8) of 4 ml elutions each.

The fractions containing protein (Figure 1 fractions 9-14 and Figure 2 fractions 9-15) were pooled and the protein concentration was determined by a Lowry assay. The concentration of the purified proteins was 5mg/ml for ATCase and 8 mg/ml for DHOase.

Lowry Assay

A Lowry assay was preformed to determine the concentration of each enzyme. ATCase had a concentration of 5 mg/ml and DHOase had a concentration of 8mg/ml.

Native Gel

Native gel electrophoresis was preformed determine the formation of the ATCase DHOase complex. This is shown in figure 3. A stable ATCase DHOase complex was observed in Aquifex aeolicus. [23] However, there is no complex formation in Bacillus anthracis.

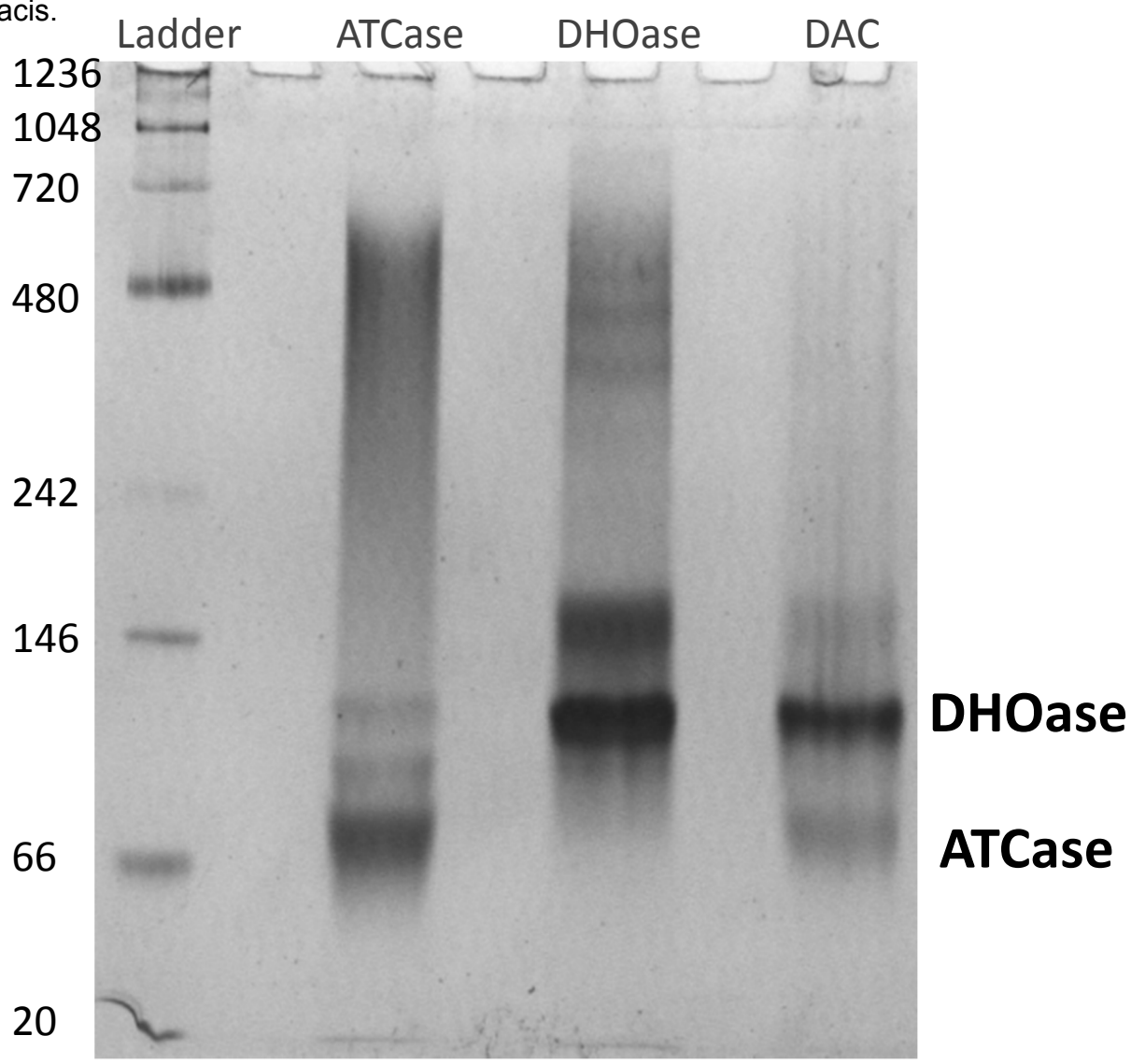

Figure 3. Native gel of native ladder, ATCase, DHOase, and DAC. The lane labeled DAC consisted of equimolar mixture of purified ATCase 10μg and DHOase 10μg.

DAC showed sample bands corresponding o both ATCase and DHOase and no higher molecular weight species suggesting that these proteins do not associate to form a complex.

Enzymatic Activity of ATCase and DHOase

An assay was performed to determine each enzyme's activity. A standard curve of differing concentrations of carbamoyl aspartate was also generated. The activity of each protein was determined using multiple concentrations of enzyme. ATCase was found to have a specific activity of about 10 μmol/min/mg. DHOase was found to have a specific activity of 5 μmol/min/mg. Saturation curves were produced using increasing concentrations of substrate. The ATCase saturation curve was fit to a Michaelis Menten equation and was found to have a Km value of 1.40 ± 0.50 and a Vmax value of 15.78 ± 2.7. The DHOase was also fit to a Michaelis Menten equation and the Km was found to be 1.09 ± 0.31 and a Vmax value of 8.07 ± 0.60. The saturation curve of ATCase is shown in Figure 4. The saturation curve of DHOase is shown in Figure 5.

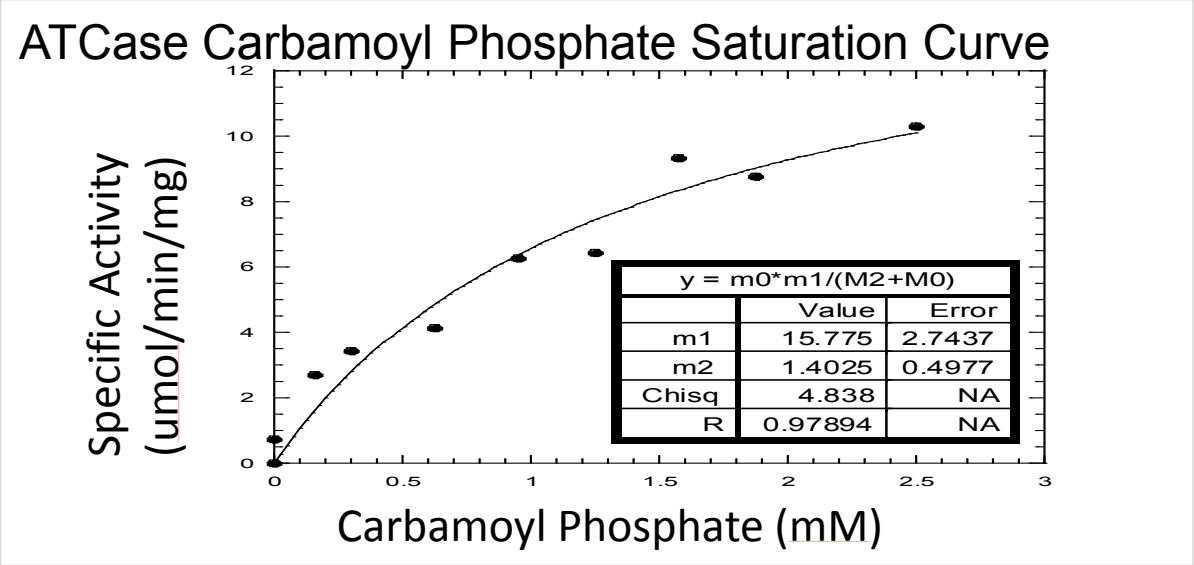

Figure 4. Saturation curve of ATCase with increasing concentrations of substrate, carbamoyl phosphate.

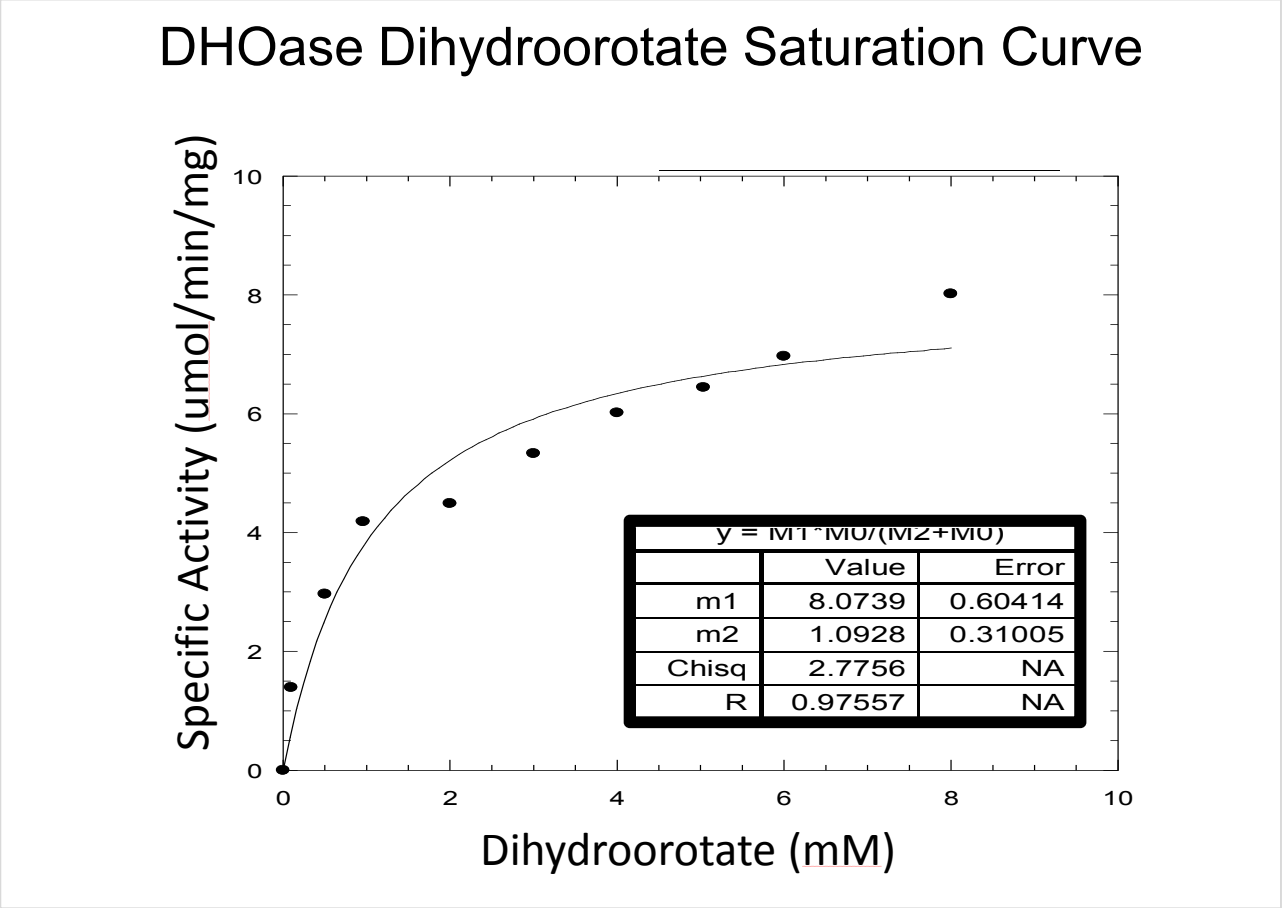

Figure 5. Saturation curve of DHOase with increasing concentrations of substrate, dihydroorotase.

The assay was then repeated using 5000umol carbamoyl aspartate and 8000umol dihydroorotate. Varying amounts of one enzyme was added to consistent volumes of the other constant. The titrations were performed in 1:0.2, 1:0.4, 1:0.6, 1:0.8, 1:1, 1:2, 1:3, 1:4, and 1:5 ratios. The titration of ATCase with DHOase is shown in figure 6. The titration of DHOase with ATCase is shown in figure 7. Activity does not significantly increase, which shows that the two do not form a complex or if complex formation occurs, it has no effect on activity.

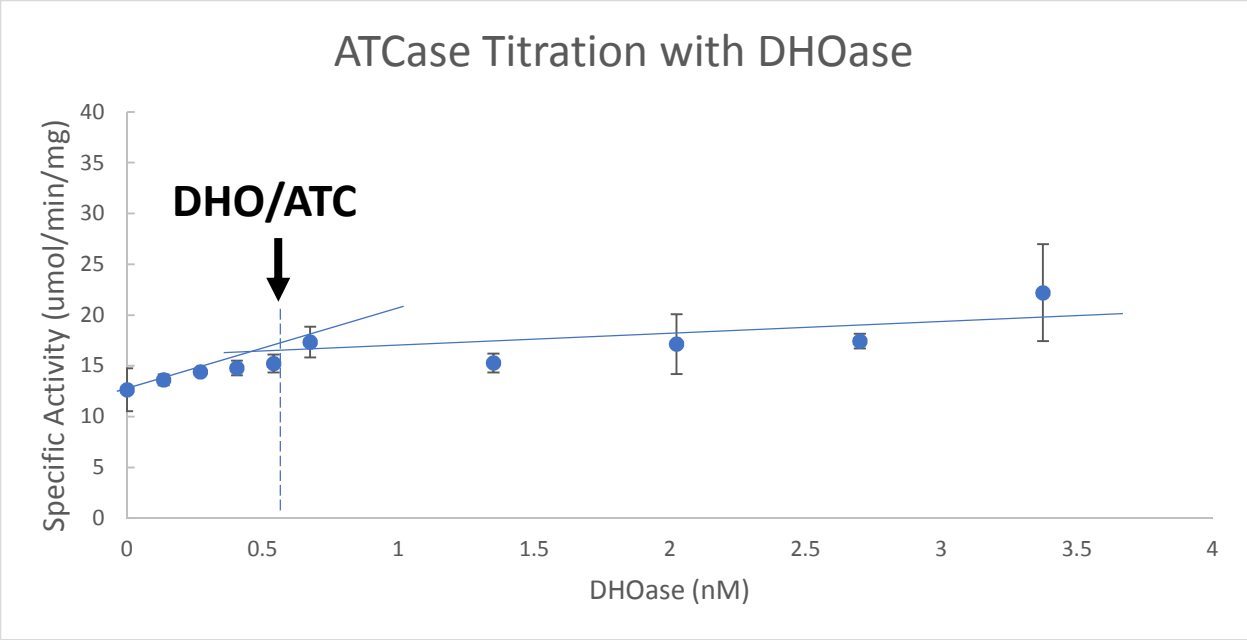

Figure 6. Titration of ATCase with differing amounts of DHOase in the presence of 5 mmol carbamoyl aspartate.

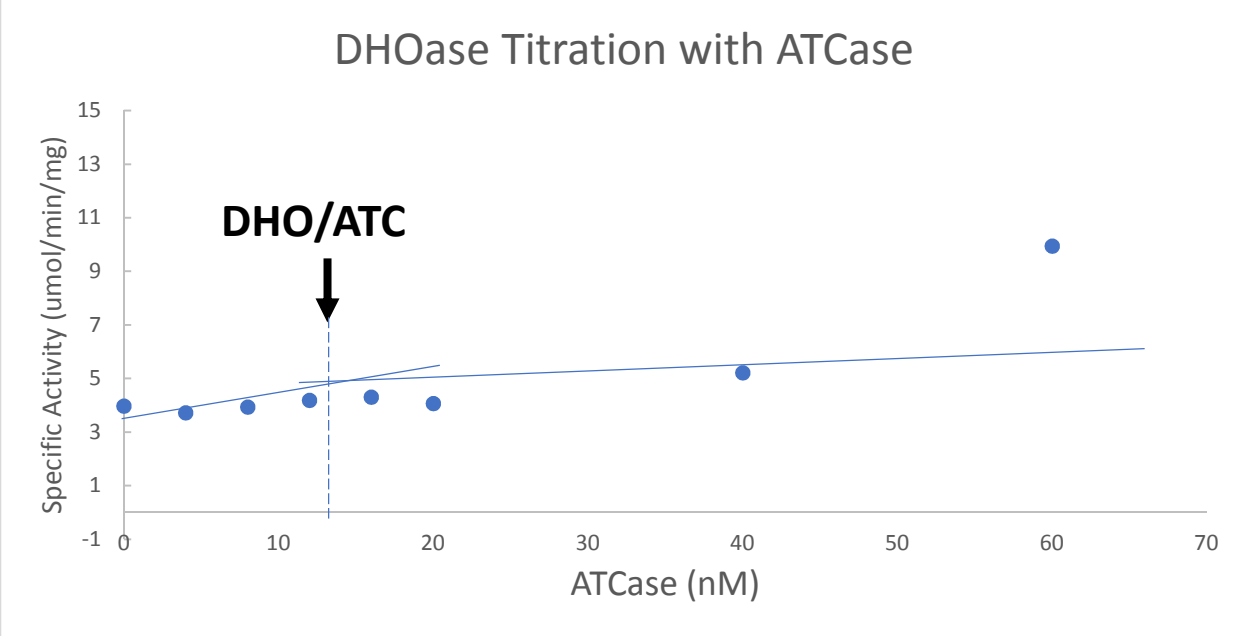

Figure 7. Titration of DHOase with differing amounts of ATCase in the presence of 8 mmol dihydroorotate.

Thermal Shift Assay

A thermal shift assay was done to detect the proteins melting temperature to assess its thermal stability. A ratio of 1:1, SYPRO reagent to protein provided the best melt curve. The derivative of the melt curve for ATCase is shown in Figure 8. The derivative of the melt curve for DHOase is shown in Figure 9. The melting temperature for ATCase was 74°C. The melting temperature for DHOase was 63°C. The DHOase ATCase (DAC) mixture had a melting point of 72°C, showing that it is ATCase dominant and is actually less stable than the enzymes alone. This shows that the mixture is not stable and therefore does not form under normal conditions. The thermal shift for the ATCase DHOase mixture (DAC) is shown in Figure 10.

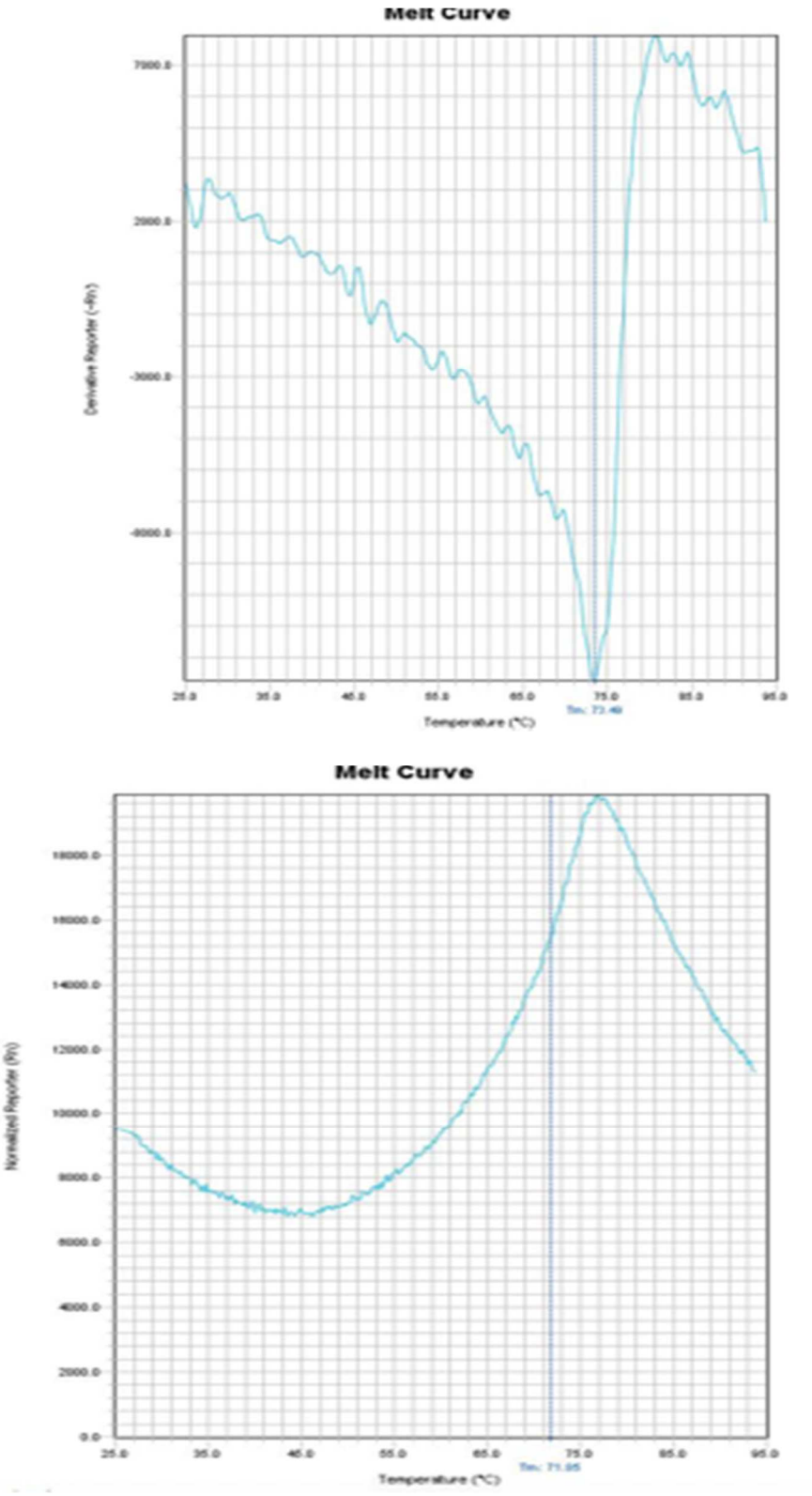

Figure 8. Thermal shift assay results of the melting curve derivative and melting curve temperature for ATCase.

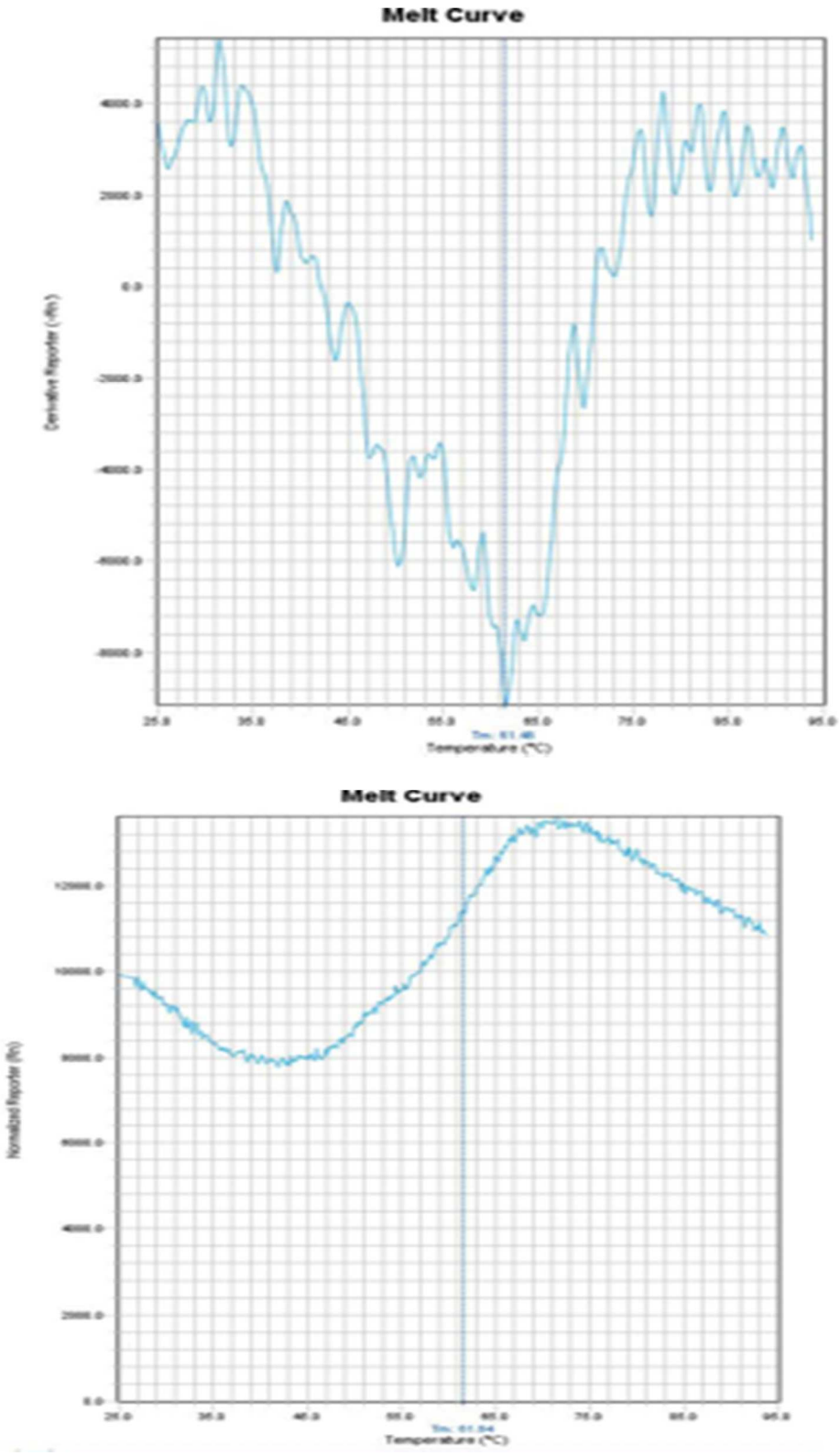

Figure 9. Thermal shift assay results of the melting curve derivative and melting curve temperature for DHOase.

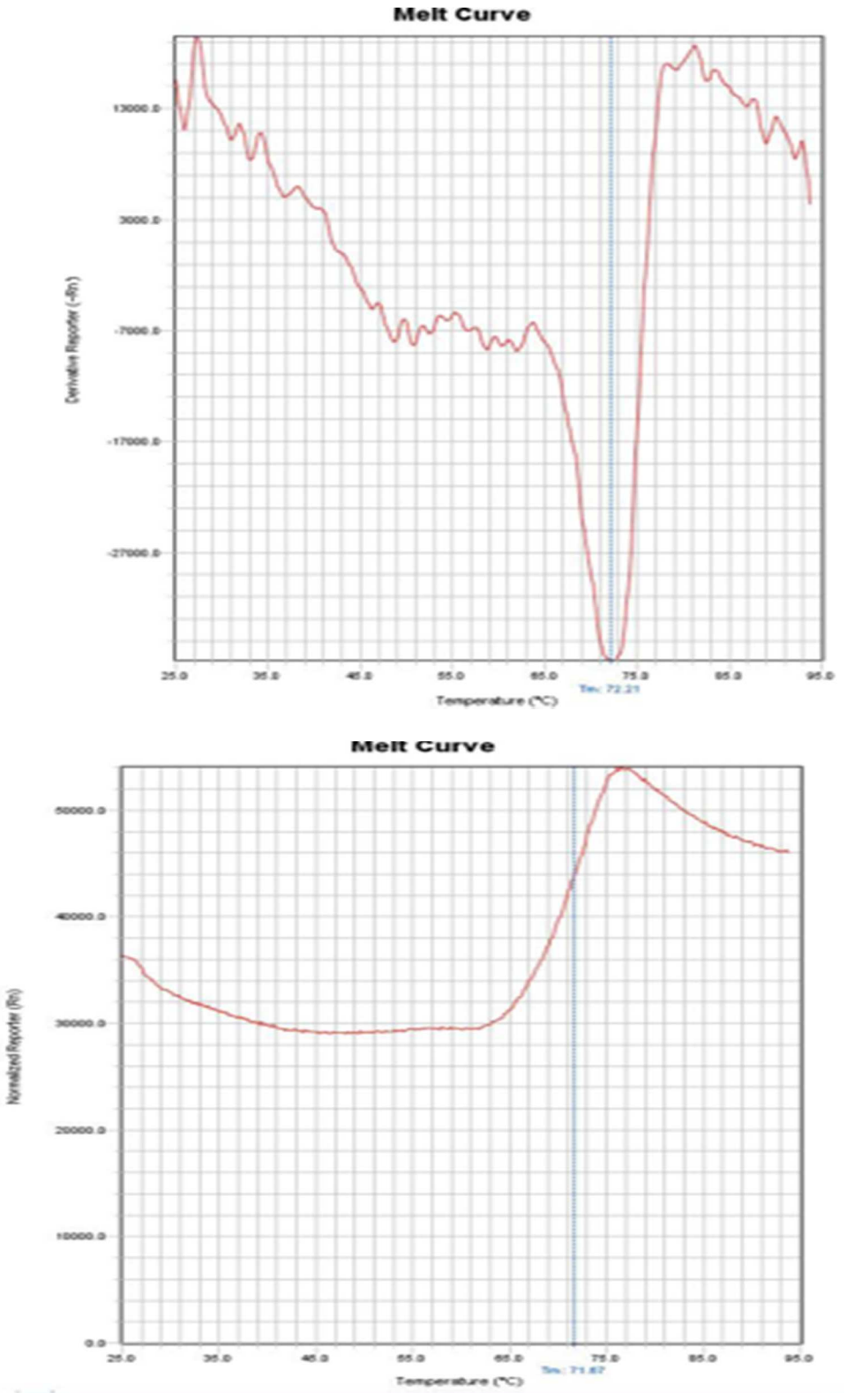

Figure 10. Thermal shift assay results of the melting curve derivative and melting curve temperature for the ATCase DHOase (DAC) mixture.

Effect of Nucleotides on *Bacillus anthracis* Inhibition Assay

The colorimetric assay described above was used to screen inhibitors such as nucleotides. Differing concentrations of the nucleotides ATP, GTP, CTP and TTP were added to a consistent concentration of enzyme. The effects of nucleotides on ATCase are shown in figures 11a through 11d. The effects of nucleotides on DHOase is shown in figures 12a through 12d. All the nucleotides tested (ATP, GTP, TTP, and CTP) show no effect on activity.

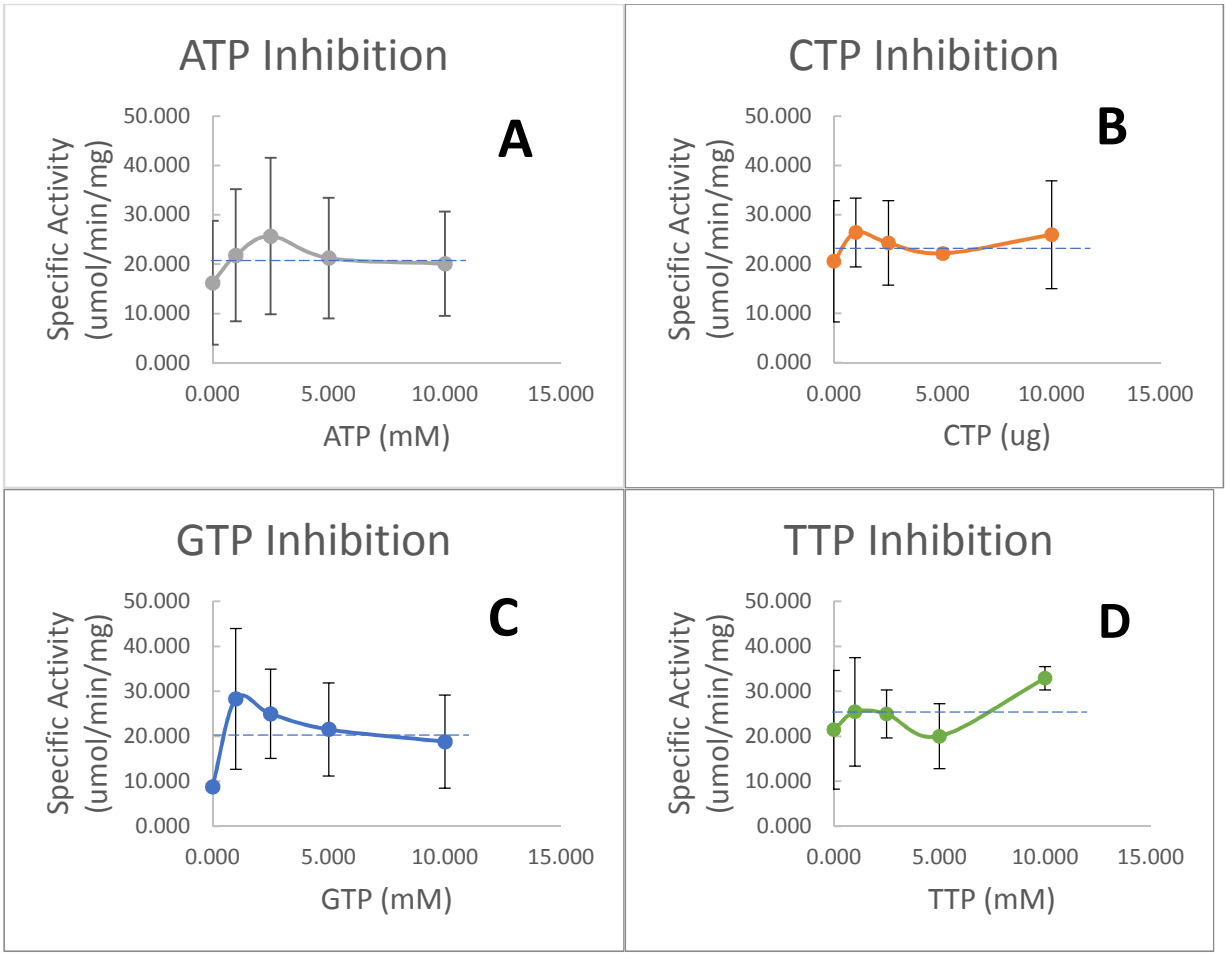

Figure 11a-11d. Activity assay showing the effects of ATP, GTP, CTP, and TTP on ATCase respectively.

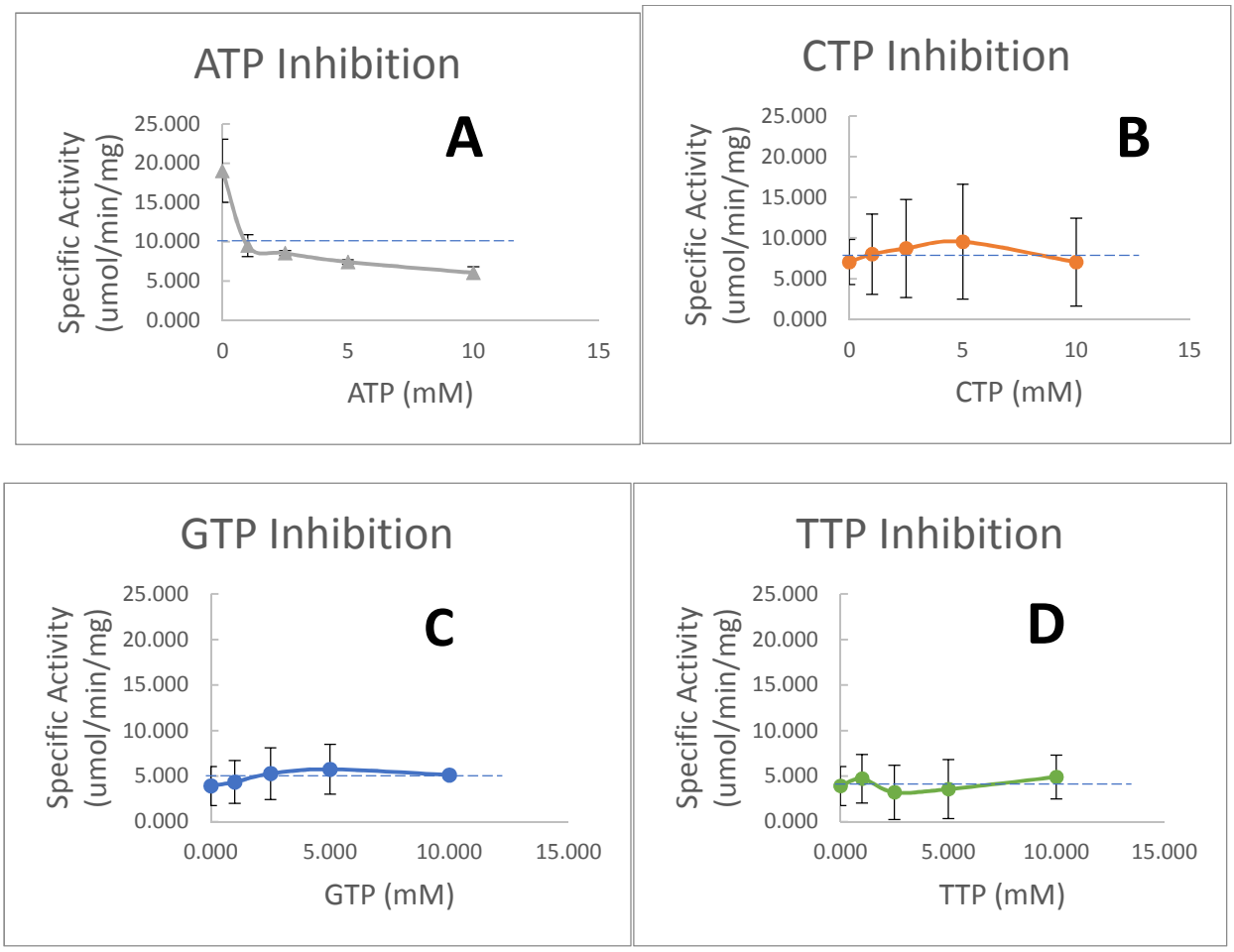

Figure 12a-12d. Activity assay showing the effects of ATP, GTP, CTP, and TTP on DHOase respectively.

Protein Characterization and Determination

Gel filtration on a 200-S column Akta filtration machine was used to determine the oligomeric structure of ATCase, DHOase, and their mixture. Gel filtration is a type of native or nondenaturing filtration. So, the proteins can be visualized in their natural oligomeric state. SDS- PAGE can be used to visualize the individual monomeric proteins or subunties because the SDS acts as a denaturing agent, breaking down the protein to its primary structure. A mixture of standard molecules (vitamin B12, myoglobin, ovalbumin, γ-globulin, and thyroglobulin) was run on the system, giving a standard linear

equation of y=-23.062x+114.5. This was used to calculate the molecular weight of the enzymes. Previous studies have suggested that ATCase and DHOase form a complex, yet they do not rely on each other for catalytic activity. [24] ATCase as a monomer has a molecular weight of 35 kDa. The molecular weight of ATCase was found to be about 89 kDA, so that the ratio of oligomer/monomer (89/35) is 2.54 suggesting that ATCase is a trimer. The ATCase peak is shown in Figure 13. DHOase as a monomer has a molecular weight of 47kDa. The molecular weight of DHO was found to be 76.5 kDa indicating that it is a dimer. The DHOase peak is shown in Figure 14. The peak of the DAC mixture is shown in Figure 15. When the two enzymes were loaded together on the gel filtration system, there is a peak at the same position where DHOase alone or ATCase alone would elute. The mass of the ATCase timer and DHOase dimer is 89 kDa and 77 kDa respectively, so the peaks are for the most part superimposed. However, there is no high molecular weight species corresponding to a higher oligomer kDa. When calculated, the molecular weight was 76 kDa, which is close to the molecular mass of the two enzymes', assuming that the ATCase and DHOase form trimers and dimers respectively. If it formed a complex there would be a narrow peak at a slightly higher molecular weight of at least 166 kDa. This result provides further evidence suggesting that the two proteins do not form a complex.

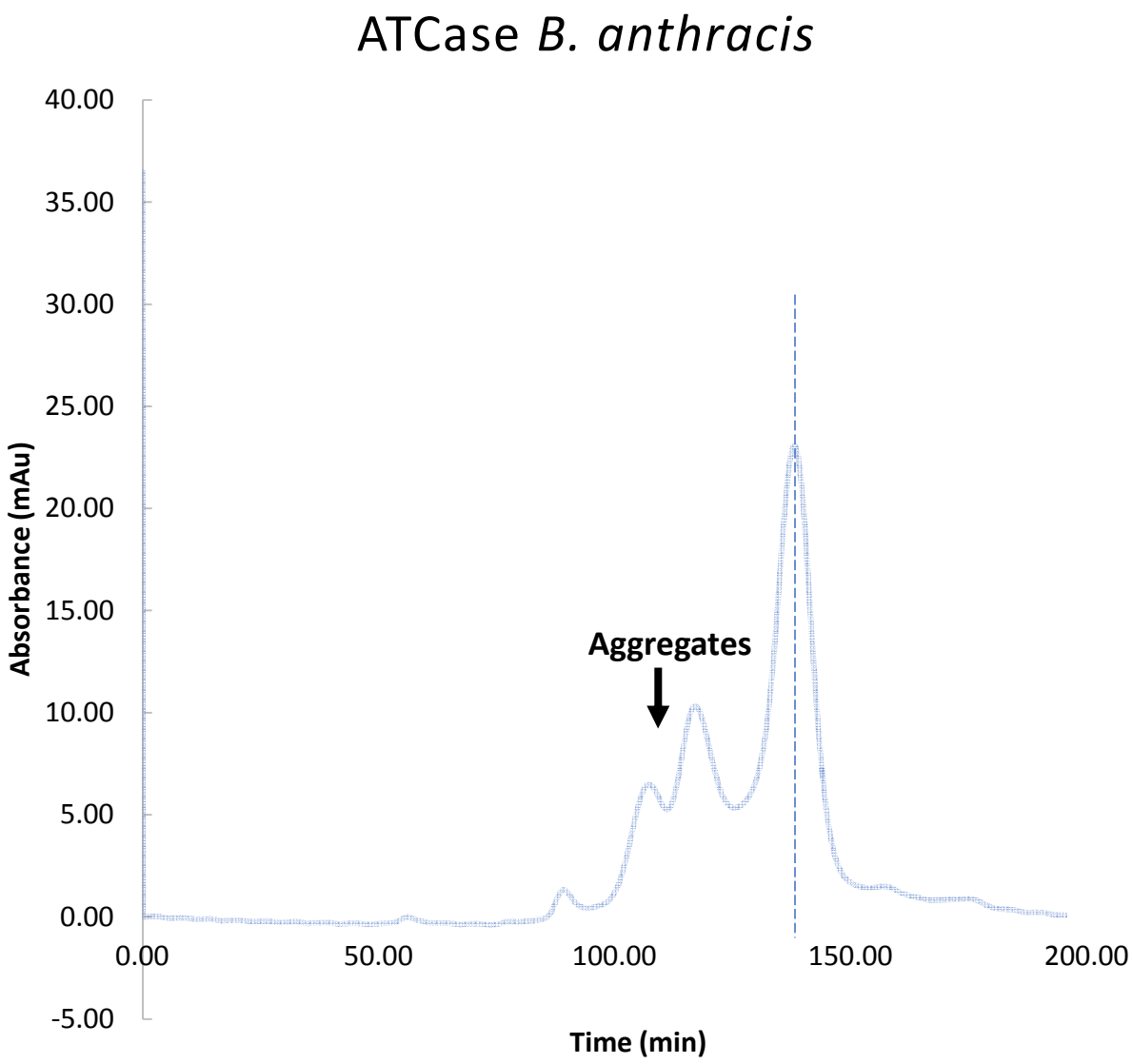

Figure 13. Gel filtration peak of ATCase.

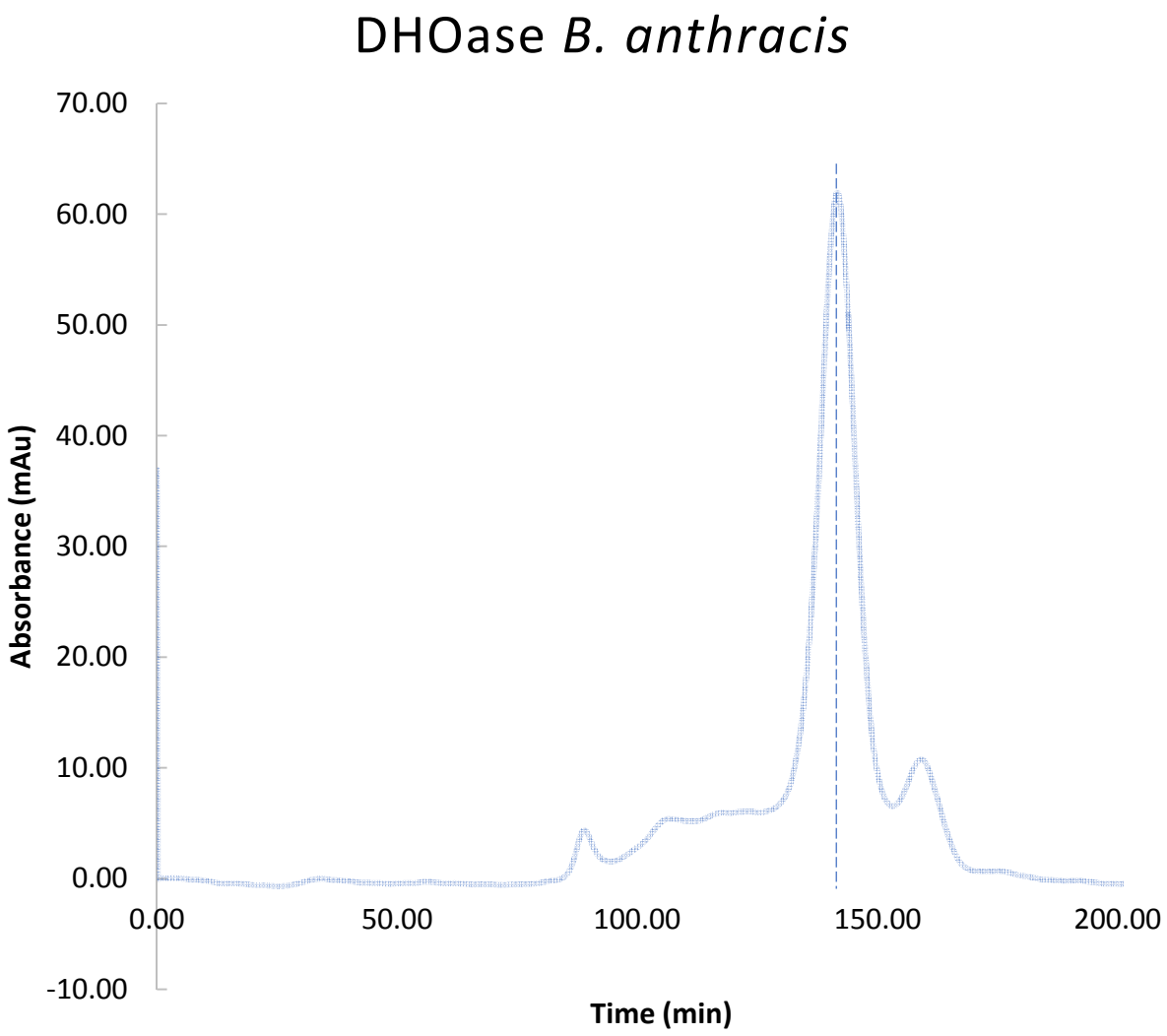

Figure 14. Gel filtration peak of DHOase.

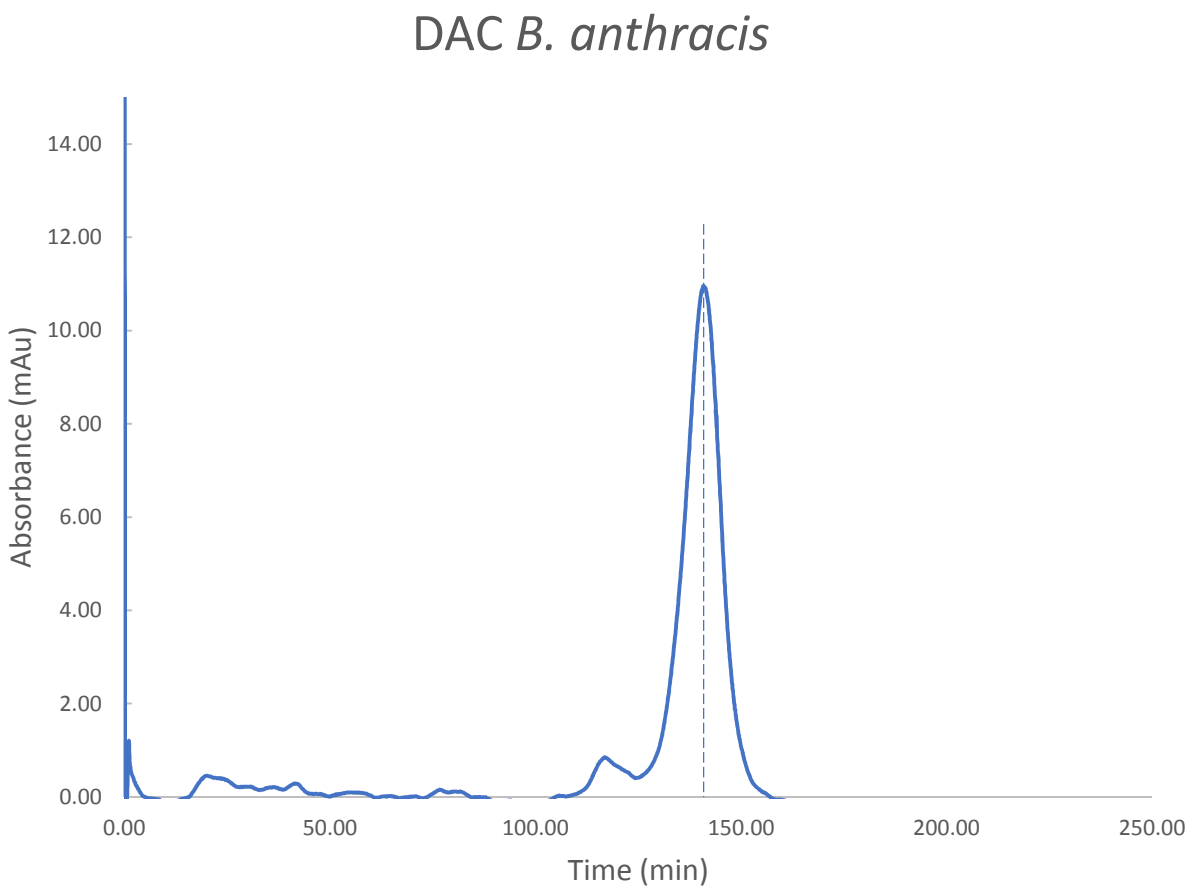

Figure 15. Gel filtration peak of DAC mixture.

CHAPTER 5: CONCLUSIONS

The equimolar mixture between *Bacillus Anthracis* aspartate transcarbamoylase and dihydroorotase slightly increased activity as opposed to the two enzymes separately. All nucleotides had little to no effect on ATCase or DHOase activity. ATCase had a melting temperature of 74°C. DHOase had a melting temperature of 63°C. The complex DAC had a melting temperature of 72°C. Gel filtration provides further evidence that unlike other systems, the ATCase and DHOase probably do not associate to form a stable complex. ATCase was found to have a molecular weight of about 37 kDa but when

chromatographed on a size exclusion column, it showed a molecular weight of about 80 kDa showing that it is a trimer. DHOase was found to have a molecular weight of about 45 kDa but gel filtration gave a molecular weight of about 90 kDa showing that it is a dimer. Both ATCase and DHOase are active in *Bacillus anthracis*, unlike that of *Pseudomonas aeruginosa* which exhibits a pseudo DHOase. The structure of DHOase in *Bacillus anthracis* has already been determined, but future work should be carried out to crystalize and determine the 3D structure of ATCase.

APPENDIX

All research was performed through Wayne State University School of Medicine.

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ABSTRACT

FUNCTIONAL ANALYSIS OF *Bacillus anthracis* ATCASE AND DHOASE

by

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Major: Biochemistry, Microbiology, and Immunology

Degree: Master of Science

There are many enzymes required for efficient and proper pyrimidine biosynthesis. The two that are most important and were discussed in this thesis are aspartate transcarbamoylase (ATCase) and dihydroorotase (DHOase). Both play an important role in not only pyrimidine biosynthesis production, but also mechanistic regulation of de novo synthesis. Anthrax is an infection caused by *Bacillus anthracis*. Here we studied ATCase and DHOase in *Bacillus* Anthracis. In this thesis we understood the effects of the enzymes ATCase and DHOase on pyrimidine biosynthesis. Adequate inhibitors of these enzymes would result in cell death and could pose as a cure to infection by *Bacillus Anthracis* or anthrax. This research showed the effects of inhibitors on the thermal stability, binding ability, and activity of the two enzymes alone. Specifically, we saw that ATCase and DHOase do not form a complex. Nucleotides had little regulatory properties on ATCase or DHOase. The biochemical and structural aspects given by these enzymes is important to human health and will lead to a better understanding of diseases such as bacteremia and anthrax.

AUTOBIOGRAPHICAL STATEMENT

In 2017, I earned a Bachelor of Science degree in Biochemistry from Oakland University in Rochester, Michigan. There, I have done research in three different laboratories, and have worked on four different projects. My first project was in Alzheimer's disease research; my second project was through NASA under the Michigan Space Grant Consortium in cataracts disease research. Both projects were done in the same biochemistry laboratory at Oakland University. My third project was in cancer imaging and diagnosing agents. This was done in an inorganic chemistry laboratory. I have given many presentations, both oral and poster, on my research of these three projects. I also have had experience teaching, as I was a TA for Chemistry and Biochemistry laboratories at Oakland University. After graduating from Oakland University, I went on to continue my education in the Biochemistry, Microbiology, and Immunology graduate program at Wayne State University School of Medicine in Detroit, Michigan. My fourth project was in Dr. Bharati Mitra's lab. My research here involved understanding how metal homeostasis is maintained in the cell, thus providing insight into the causes and potential cures to metal transporter associated diseases, such as cancer. My fifth project was with Dr. David Evans in determining the activities of ATCases and DHOases. This research was done at Wayne State University School of Medicine and is the basis of this thesis. After graduation, I plan to pursue my education by obtaining a PhD and then to find a job in industry or the government. Apart from science, my hobbies include music and traveling.