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**Optimal Biochemical Conditions and Inhibition Analysis of *Klebsiella pneumoniae*
Inorganic Pyrophosphatase**

by

Christopher Otto Sebastian

An Honors Thesis

submitted in partial fulfillment of the requirements

for the Honors Diploma

to

The Honors College

of

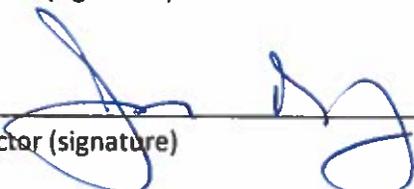
The University of Alabama in Huntsville

1 May 2017

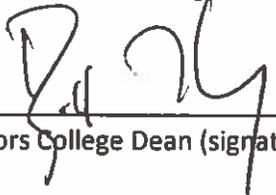
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Lab of Dr. Joe Ng

Optimal Physical Condition and Inhibition Analysis of *Klebsiella pneumoniae* Inorganic Pyrophosphatase

Christopher Sebastian

Lab of Dr. Joe Ng

Abstract

***Klebsiella pneumoniae* is a member of a group of opportunistic pathogens known as ESKAPE pathogens, which are known hospital-acquired infections that have strains that have developed a resistance to known antibiotics. Inorganic Pyrophosphatase is an essential, metal-dependent enzyme ubiquitous to all life that catalyzes the hydrolysis of inorganic pyrophosphate ions into two inorganic phosphate ions. In the present study, we prepared an assay to run the reaction at controlled temperature and pH coupled with an ammonium molybdate/sulfuric acid colorimetric reaction to determine the concentration of phosphate formed during the reaction to determine the optimal pH of 7.0, temperature of 55 °C, and divalent cation of magnesium necessary for enzymatic activity. Additional studies using an unknown compound to determine a potential inhibitor found significant inhibition, whose impact could lead to the development of a novel antibiotic previously undiscovered. Structural speculations were additionally made in order to understand some of the physical characteristic of the enzyme.**

Introduction

Klebsiella pneumoniae is a gram negative, rod shaped bacterium classified as a nosocomial infection and is an ESKAPE pathogen (1). ESKAPE pathogens constitute many of the known nosocomial infections, including *Enterococcus faecium*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter sp.*, and *Klebsiella pneumoniae*. Many strains have developed resistance to known antimicrobial agents. For example, *Klebsiella pneumoniae* contains strains resistant to aminoglycoside, fluoroquinolone, tetracycline, chloramphenicol, and trimethoprim/sulfamethoxazole (2). Thus, molecular targeting of various enzymes vital to the proper function of the organisms physiology, such as Inorganic Pyrophosphatase, has become incredibly important in the development of novel therapeutic agents.

Inorganic Pyrophosphatase ((EC 3.6.1.1., IPPase) is an essential, metal-dependent enzyme that is associated with several metabolic pathways including replication and transcription in all domains of life (3). IPPase is responsible for the hydrolysis of inorganic pyrophosphate ($P_2O_7^{4-}$, PPI) into inorganic phosphate (PO_4^{3-} , Pi)(4). The hydrolysis reaction creates a high energy discharge which provides a thermodynamic sink that drives unfavorable reactions in the forward direction (5).

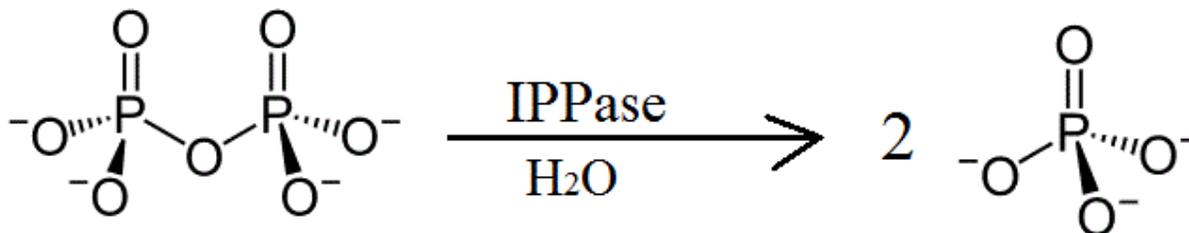


Figure 1. Hydrolysis reaction of inorganic pyrophosphates into two inorganic phosphate molecules.

IPPase can be separated into three fundamental families: Family I, Family II, and Membrane bound. In this study, only cytosolic IPPase enzymes are observed. Family I IPPase's are found in many bacteria and most eukaryotes, whereas Family II are present in only some bacteria and primitive eukaryotes (7). Family I IPPase's typically rely on magnesium ions in order to function properly, and Family II IPPase's typically rely on manganese ions (3). The structural differences between prokaryotic and mammalian Family I IPPase's provide an opportunity potential target for the development of new antibiotics.

In the present study, IPPase from *Klebsiella pneumoniae* was targeted for preliminary inhibition studies. Natural product extracts were screened for enzymatic inhibition and, out of 67 samples, one showed significant inhibition. The sample will be referred to as ELMABM in this report. The structure and concentration of the ELMABM is still undergoing study. Additional, more specific values for enzyme activity and inhibitor (K_m , K_i , and IC_{50}) cannot be provided until access to a stopflow, a piece of equipment used for enzyme kinetics, is made available. Despite those challenges, the discovery of an inhibitor for the IPPase of *Klebsiella pneumoniae* provides direction in the development of potential antibiotics.

Materials and Methods

Enzyme Activity Assay

The enzyme assay reaction solution was prepared with 30 mM of a Tris-HCl buffered to a specified pH at selected temperature, containing 7.5 mM $MgCl_2$ and 500 μM of sodium pyrophosphate. Eight tubes were prepared for each set of conditions. Six of the tubes served as the primary reaction, in which all components of the solution were present. Two tubes were labelled blank and control. The tube labelled "blank" received 1 μL of water in addition to the other components. The tube labelled "control" contained no sodium pyrophosphate; instead, an identical volume of water was placed in the solution.

All eight tubes were placed in a water bath set to a specified temperature and were allowed to incubate for five minutes. The tubes were then removed and 723 ng of *Klebsiella pneumoniae* Inorganic Pyrophosphatase was added into each tube except for the tube labelled “blank.” The reaction was placed back into the water bath and was left alone for ten minutes.

A volume of 100 μ l containing 1 g of ammonium molybdate per ml of sulfuric acid supplemented with 50 mg of Ferrous Sulfate per ml was placed into eight wells of a 96-well plate. At the end of a ten minute reaction period, the tubes were removed from the water bath and 100 μ l from each tube was added into an individual well containing this solution. The resulting reaction was allowed to proceed for five minutes. At the end of the five minutes, the plate was scanned in a spectrophotometer at a 660 nm absorbance. The data was then copied, plotted in Microsoft Excel, and compared to a phosphate standard to measure the liberation of phosphate in the reaction. The subsequent value was then used to determine the specific activity of the enzyme. In each condition set, the optimal condition was chosen from the condition that exhibited the greatest activity. All enzyme reactions were 1 ml in volume.

Optimal Temperature Assays

The enzyme assay mentioned above was used to perform these assays with a few minor differences. Eight sets of eight tubes were assayed. Each set was assigned a different temperature in which the assay would be performed, which were 35 °C, 37 °C, 40 °C, 45 °C, 50 °C, 55 °C, 60 °C, and 70 °C.

Optimal pH Assays

The enzyme assay mentioned above was used to perform optimal pH assays with a few minor differences. Nineteen sets of eight tubes were assayed. The buffers in each tube were not identical. Four of the sets of tubes contained 30 mM sodium cacodylate buffered to a pH of 5.5, 6.0, 6.5, and 7.0 respectively. Four of the sets of tubes contained 30 mM MOPS buffered to a pH of 6.5, 7.0, 7.5, and 8.0 respectively. Five of the sets of tubes contained 30 mM Tris-HCl buffered to a pH of 7.0, 7.5, 8.0, 8.5, and 9.0 respectively. Three of the sets of tubes contained 30 mM AMPPO buffered to a pH of 8.0, 8.5, and 9.0 respectively. Three of the sets of tubes contained 30 mM CHES buffered to a pH of 8.5, 9.0, and 9.5 respectively.

Optimal Metals Assays

The enzyme assay mentioned above was used to perform optimal metal assays with a few minor differences. Six sets of tubes were assayed. Only one set of tubes received 7.5 mM MgCl₂. The rest contained either 7.5 mM MnCl₂, 7.5 mM CoCl₂, 7.5 mM NiCl₂, 7.5 mM CaCl₂, or 7.5 mM ZnCl₂ respectively.

Inhibition Assays

Inhibition Assays contained additional components and controls. A set of 67 unknown compounds provided by the lab of Dr. William Setzer were screened for the potential for inhibition using Inorganic Pyrophosphatase from *Acinetobacter baumannii*. Each unknown compound was concentrated to 1% in DMSO. Of the 67 compounds, only the compound labelled ELMABM was found to inhibit the enzyme over the course of a 10 minute activity assay.

Two sets of nine tubes were prepared for each assay. Six of the tubes had unknown compound added to it (with enough water removed to keep the reaction volume at 1 ml). One set received 5 µl of the unknown compound and one set received 15 µl of the unknown compound. The regular ingredients for the enzyme activity assay was used as a positive control. Another tube had a volume of DMSO matching the volume of unknown compound added in the reaction mix with no enzyme as a control. The remainder of the enzyme activity assay was performed as stated above.

The product, ELMABM, was then assayed with the enzyme to ensure that it provided inhibition in multiple ESKAPE pathogen Inorganic Pyrophosphatases. Three individual conditions were assayed. A condition labelled the positive control was performed using the optimal conditions determined in the prior experiments. A blank containing the enzyme reaction base that did not receive enzyme and a control that did not receive pyrophosphate was assayed alongside these conditions. The second condition was labelled ELMABM, and was prepared using the same ingredients as the primary reaction with the addition of 20 µl of the product labelled ELMABM included in the enzyme reaction base. The third condition was labelled DMSO control, and was prepared using the same composition as the primary reaction with the addition of 20 µl of DMSO included in the enzyme reaction base. 6 experiments were performed for each condition.

Modelling Klebsiella pneumoniae Inorganic Pyrophosphatase

It is important to provide structural analysis of the enzyme at hand. A sequence was found using the UNIPROT website. It is important to note that the sequence of the protein used has not been reviewed, and therefore any structural discussion will be hypothetical speculations intended to explain certain chemical properties determined experimentally. The sequence from UNIPROT was then copied and pasted into SWISS model, which modelled the sequence from UNIPROT to a known structure with the highest sequence identity, which was only 43.10% (9). Although using this structure will be good for hypothetic speculations, a truly scientific structural discussion cannot take place until X-ray diffraction data has been received for *Klebsiella pneumoniae* Inorganic Pyrophosphatase and its structure determined. The models were then viewed in the program PYMOL.

Results

Optimal Temperature

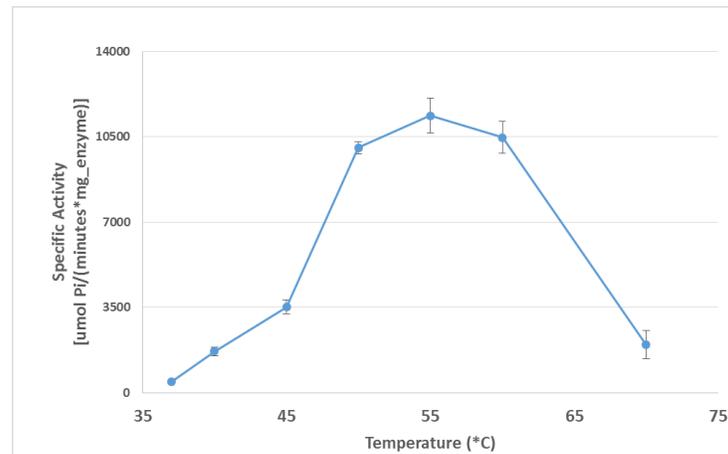


Figure 2 Optimal temperature of *Klebsiella pneumoniae* Inorganic Pyrophosphatase.

Klebsiella pneumoniae Inorganic Pyrophosphatase exhibited an optimal temperature of 55 °C. At 55 °C (Fig 2), the enzyme expressed an average specific activity amongst six repetitions of the experiment at that condition of approximately 11,400 ± 648 µmoles of phosphate liberated per milligram of enzyme a minute. At 60 °C, the enzyme exhibited approximately 10,500 ± 588 µmoles of phosphate liberated per milligram of enzyme a minute under the same conditions, coming in at the second highest specific activity. At 70 °C, which was the highest temperature the water bath used would accurately reach, exhibited approximately 1980 ± 782 µmoles of phosphate liberated per milligram of enzyme a minute. The lowest data point graphed is 37 °C, which exhibited 447 ± 176 µmoles of phosphate liberated per milligram of enzyme a minute. The absolute lowest was not included in the graph, which was 30 °C, and it experienced a negative specific activity when solved for the units provided. The control exhibited no reaction.

Optimal Divalent Cation

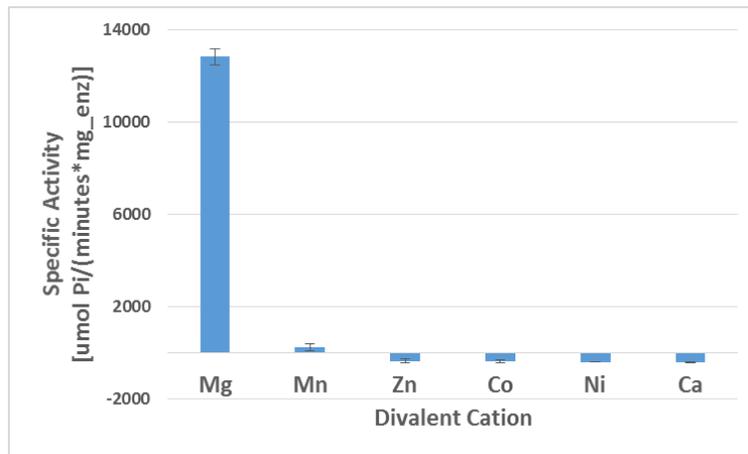


Figure 3 Optimal divalent cation of *Klebsiella pneumoniae* Inorganic Pyrophosphatase.

Magnesium was determined to be the optimal divalent cation enzyme activity (Fig 3). The specific activity in the presence of magnesium exhibited $12,800 \pm 334$ µmoles of phosphate liberated per milligram of enzyme a minute. Manganese exhibited 216 ± 157 µmoles of phosphate liberated per milligram of enzyme a minute, which is a 98.3% decrease in activity. All of the other metals examined were associated with negative specific activities, and thus we assume that there is no activity. Additionally, the control exhibited no activity.

Optimal pH

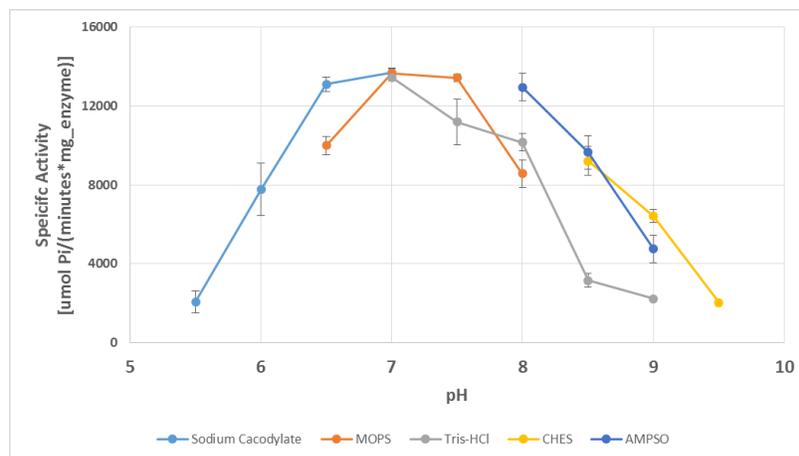


Figure 4 Optimal pH of *Klebsiella pneumoniae* Inorganic Pyrophosphatase.

The optimal pH for *Klebsiella pneumoniae* Inorganic Pyrophosphatase was determined to be a neutral pH of 7.0 (Fig 4). The three buffers assayed at a pH of 7.0 all exhibited similar activity. Using sodium cacodylate, the enzyme reaction at a pH of 7.0 exhibited a specific

activity of $13,700 \pm 244$ μ moles of phosphate liberated per milligram of enzyme a minute. Using Tris-HCl, the enzyme reaction at a pH of 7.0 exhibited a specific activity of $13,500 \pm 173$ μ moles of phosphate liberated per milligram of enzyme a minute. Using MOPS, the enzyme reaction at a pH of 7.0 exhibited $13,700 \pm 210$ μ moles of phosphate liberated per milligram of enzyme a minute. The assay using Tris-HCl at a pH of 7.0 was slightly lower in activity than the assays using MOPS and sodium cacodylate, with a 1.46% decrease in activity. Using MOPS at a pH of 7.5, the specific activity was $13,400 \pm 199$ μ moles of phosphate liberated per milligram of enzyme a minute, which is a 2.19% decrease in activity from using MOPS at a pH of 7.0. The enzyme reaction using sodium cacodylate at a pH of 6.5 exhibited a specific activity of $13,100 \pm 359$ μ moles of phosphate liberated per milligram of enzyme a minute, which is a 4.37% decrease in activity. Beyond that range of pH, the specific activity of the enzyme reaction decreases dramatically.

Inhibition Analysis

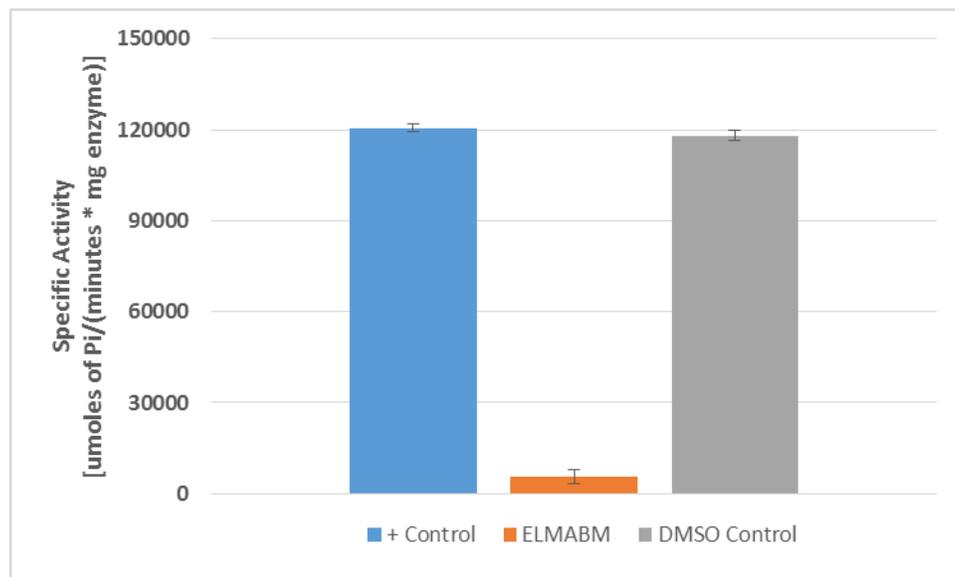


Figure 5 Inhibition Assay of *Klebsiella pneumoniae* using ELMABM.

The positive control condition experiments exhibited a specific activity of $12,100 \pm 127$ μ moles of phosphate liberated per milligram of enzyme a minute. The DMSO control experiment exhibited $11,800 \pm 168$ μ moles of phosphate liberated per milligram of enzyme a minute, which is a 2.47% decrease in activity in comparison to the positive control experiment. The set of experiments that contained 20 μ l of the product labelled ELMABM exhibited only 574 ± 231 μ moles of phosphate liberated per milligram of enzyme a minute, which is a 95.1% decrease in activity in comparison to the DMSO control and 95.3% decrease in activity in comparison to the positive control.

Model of Klebsiella pneumoniae Inorganic Pyrophosphatase

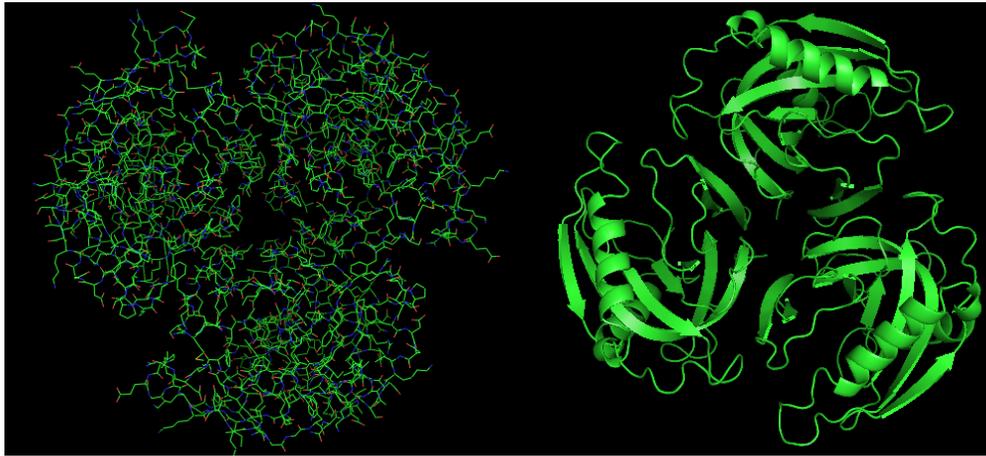


Figure 6. *Klebsiella pneumoniae* Inorganic Pyrophosphatase model. Left is a stick model, while the right is a ribbon model.

10	20	30	40	50
MHLVKTILTA	GLLLSAAAQA	HNVLEFPQPE	NNPEEFYAVT	EIPTGGIIKY
60	70	80	90	100
ETDAKTGFIV	ADRFQSMPVA	YPANYGSLTQ	SLAGDGDPLD	VVFYTRAPMA
110	120	130	140	150
PGTLLIKLRAI	GVLKMIDGGE	KDDKIIAVPA	SKIDPTYDDI	KTISDLPKIE
160	170	180	190	
QQRLEAFFRV	YKELPEGRKK	VELAGFNDA	AAKQEIKSAW	EAWKAKNPQ

Figure 7. The sequence of *Klebsiella pneumoniae* Inorganic Pyrophosphatase used from UNIPROT (10).

The model in figure 5 was prepared from a known structure of *Litopenaeus vannamei* Inorganic Pyrophosphatase, sharing only 43.10% sequence identity. The structure is a homotrimer connected in the center by an OB-fold. Figure 6 shows the sequence, which is 199 amino acids in length. Active site residues are residues 49, 63, 75, 85, 90, 122, and 163. Sites 85 and 90 are listed to bind magnesium ions, whereas sites 49, 63, 75, 122, and 163 are listed to bind substrate (inorganic pyrophosphate). Sites 85 and 90 are aspartic acid residues, which are negatively charged. Site 49 is a lysine residue. Site 63 is an arginine residue, which is positively charged. Site 75 is a tyrosine residue, whose side chain has polarity, but is assigned a formal charge of zero. Site 122 is an aspartic acid residue and site 163 is a glutamic acid residue, both of which are negatively charged residues. Outside of the active site, there are thirteen negatively charged glutamic acid residues eleven negatively charged aspartic acid residues, sixteen positively charged lysine residues, five positively charged arginine residues, and two positively charged histidine residues, providing twenty five total negatively charged residues and twenty three positively charged residues. This gives a total formal charge of -2 on the protein if you exclude the active site and a total formal charge of -3 if you include the active site. An additional thirty-six residues with polar side chains were counted that were not included in the active site.

Discussion

Collectively, the optimal biochemical characteristics of *Klebsiella pneumoniae* Inorganic Pyrophosphatase determined in the study was a temperature of 55 °C, an optimal pH of 7.0, and an optimal divalent cation of Magnesium.

The optimal temperature range was expected to be between 30 °C and 40 °C. *Klebsiella pneumoniae* has been found to grow optimally and equivalently from a range of 18 °C to 47 °C, but is known to grow at temperatures higher than that, with growth inhibition not occurring until a temperature closer to 50 °C, where it was found to die within 30 minutes of exposure (8). The finding that *Klebsiella pneumoniae* Inorganic Pyrophosphatase had an optimal temperature of 55 °C was initially unexpected. The experiments were repeated three times before finally concluding that this was the case. As mentioned before, *Klebsiella pneumoniae* is an organism that grows well at 47 °C, and at 50 °C Inorganic Pyrophosphatase showed a significantly high level of activity.

As experimentally determined in the present laboratory, Inorganic Pyrophosphatase from a mesophilic organism typically has an optimal range between 30 °C and 40 °C. Thus a structural speculation was prepared in order to understand why it is that *Klebsiella pneumoniae* Inorganic Pyrophosphatase experiences such optimal activity at such a high temperature and so little activity at such a low temperature *in vivo*. Furthermore, experimental evidence of Inorganic Pyrophosphatase from other mesophilic organisms reviewed by this lab has shown that activity degrades rapidly beyond 40 °C. The initial hypothesis was that the enzyme was supplemented with disulfide bridges throughout its structure; however, upon reviewing the sequence it was determined that no disulfide bridges were present due to the lack of cysteine residues.

The next hypothesis was determined by viewing the structure of Inorganic Pyrophosphatase from the thermophilic microorganism *Thermus thermophilus*. In the thermophilic Inorganic Pyrophosphatase, the trimeric structure was found to have a decrease in 16% surface area in comparison to Inorganic Pyrophosphatase from *Escherichia coli*. The decrease in surface area results from the monomers being 0.3 Å closer to the center as well as other changes in the conformation in the overall protein. The thermophilic Inorganic Pyrophosphatase additionally contained much more hydrogen and ionic bonding covering the surface of the molecules than does the *Escherichia coli* Inorganic Pyrophosphatase, and thus that would increase the stability of the protein in the presence of greater thermal energy.

10	20	30	40	50
MSLLNVPAGK	DLPEDIYVVI	EIPANADPIK	YEIDKESGAL	FVDRFMSTAM
60	70	80	90	100
FYPCNYGYIN	HTLSLDGDPV	DVLVPTPYPL	QPGSVIRCRP	VGVLKMTDEA
110	120	130	140	150
GEDAKLVAVP	HSKLSKEYDH	IKDVNDLPEL	LKAQIAHFFE	HYKDLEKGGK
160	170			
VKVEGWENAE	AAKAEIVASF	ERAKNK		

Figure 8. Sequence of inorganic pyrophosphatase from *Escherichia coli* (12).

Comparing the sequence of *Escherichia coli* Inorganic Pyrophosphatase to the sequence of *Klebsiella pneumoniae* Inorganic Pyrophosphatase, we find that there is an equivalent formal charge when including all charged residues, but that there are ten fewer polar residues in *Escherichia coli* Inorganic Pyrophosphatase. Although the precise location of the additional polar residues in *Klebsiella pneumoniae* Inorganic Pyrophosphatase is not known, we can hypothesize that these additional residues will provide a source of hydrogen bonding across other residues along the surface of the monomers.

Additionally, the hypothesis includes a decrease in overall surface area in *Klebsiella pneumoniae*, which would create a greater effect in the level of hydrogen bonding across the surface. Of course, the decrease in overall surface area could be a result of hydrogen bonding as well. The answer to such a question can be concluded upon determination of the crystallographic structure of *Klebsiella pneumoniae* Inorganic Pyrophosphatase.

The protein additionally exhibits significantly less activity at 37 °C as it does at 55 °C, and at temperatures below that there is essentially no activity; however, we know that the reaction catalyzed by Inorganic Pyrophosphatase is essential to life since pyrophosphate acts as a negative allosteric regulator amongst many proteins within the cell, a significantly high concentration of which would cause cell death. Thus, we must assume that the enzyme is active in the cell even at 18 °C, which was reported earlier to show the same growth as if it the organism were grown at 37 °C or 47 °C. How exactly this occurs is unknown. Approaching this would likely require an *in vivo* analysis as opposed to an *in vitro* analysis as done in this study. For example, a trace fluorescence label could be tagged to the enzyme and the level of movement viewed while in the cell. Additionally, if the enzyme is only present at certain temperatures, this would lead to the conclusion that the organism utilized multiple Inorganic Pyrophosphatase enzymes. Neither of these cases have been experimentally determined, and should be viewed purely as speculation.

Klebsiella pneumoniae has a physiological pH of 7.2 (13); however, the organism was not found to have significant growth inhibition at a wide variety of pH's (8). Thus, the initial hypothesis for the optimal pH for the activity of *Klebsiella pneumoniae* Inorganic Pyrophosphatase was speculated to range from 6.5 to 8.0. Experimental data determined that the hypothesis was correct, with very little difference in activity between the four pH levels. At a pH of 8.0, however, the enzyme is dependent upon the use of AMPSO as a buffer, whereas other

buffers at that pH show significant decreased activity at a pH of 7.0, which was determined to be the optimal pH. To understand this, an analysis of the pKa of the buffers AMPSO, Tris-HCl, and MOPS were compared. AMPSO was found to have a pKa of 9.0 at 25 °C and optimal pH buffering capacity between 8.3 to 9.7, Tris-HCl was found to have a pKa of 8.08 at 25 °C with an optimal pH buffering capacity between 7.0 and 9.0, and MOPS was found to have a pKa of 7.2 with an optimal pH buffering capacity between 6.5 and 7.9 (14,15). Using MOPS, a pH of 8.0 is just barely beyond the capacity of the buffer, and thus it is assumed for the purposes of this discussion that the solution suffered no significant and unmeasurable change in pH during the reaction. The basis for this assumption is on the comparison of the reaction using MOPS with the reaction using Tris-HCl, where the buffering capacity is strongest around a pH of 8.0. Both show decreased activity. The buffering capacity of AMPSO is 0.3 pH units higher than the pH of the solution, and thus it is possible that the pH of the solution fell below 8.0. Compared to the activity at 7.0 and 7.5, this would lead to greater catalytic activity by the enzyme.

The initial hypothesis on the optimal divalent cation was presumed to be magnesium on the basis that *Klebsiella pneumoniae* uses Family I Inorganic Pyrophosphatase enzymes, which experimental evidence has shown to be true. Family II Inorganic [yrophosphatase enzymes are manganese dependent, and thus there was a curiosity as to whether or not manganese would exhibit any activity in *Klebsiella pneumoniae* Inorganic Pyrophosphatase. Experimentally, it was found that there was some activity in the presence on manganese; however, there was a significant drop in activity. Other metals were assayed to determine if there was any activity mostly for curiosity's sake, to which we determined that there was no effect.

Analysis of ELMABM used with the solution in which the reaction occurs shows significant inhibition. The ELMABM was prepared as 1% in DMSO, and thus DMSO was assayed to ensure that DMSO was not the inhibiting component, and thus it can be inferred the ELMABM is a potent inhibitor of *Klebsiella pneumoniae* Inorganic Pyrophosphatase. The specific structure of ELMABM is unknown, and furthermore it is unknown how many different components are in the solution. Studies will be performed by crystallizing the product with the enzyme to see the interactions of the compound with the enzyme. Further studies to separate the components and determine the structure of the component will be performed. Additionally, we will assay the component with *Homo sapien* Inorganic Pyrophosphatase to determine if there is similar inhibition. The overall structure of the two enzymes are different with highly conserved active sites over the course of evolution. If there is component-enzyme interactions at the connection sites of the monomers or allosteric regulation of the active site in the *Klebsiella pneumoniae* Inorganic Pyrophosphatase that does not occur in *Homo sapien* Inorganic Pyrophosphatase, then this component will be a great compound for use in the development of a novel antibiotic. However, if the component causes significant inhibition in *Homo sapien* Inorganic Pyrophosphatase, we assume that there is some interaction directly with the active site or the substrate that prevents the enzyme from performing its catalytic activity.

References

- 1) Rice, LB. "Progress and challenges in implementing the research on ESKAPE pathogens." *Infect Control Hosp Epidemiol*. 2010 Nov;31 Suppl 1:S7-10. doi: 10.1086/655995.
- 2) Nathisuwan, S; *et al.* "Extended-spectrum β -Lactamases: Epidemiology, Detection, and Treatment". *Pharmacother*. Aug 2001. 21(8). P. 920-8. Doi: 10.1592/phco.21.11.920.34529
- 3) Gajadeera, Chathurada S., *et al.* "Structure of inorganic pyrophosphatase from *Staphylococcus aureus* reveals conformational flexibility of the active site." *Journal of structural biology* 189, no. 2 (2015): 81-86.
- 4) Harold FM (December 1966). "Inorganic polyphosphates in biology: structure, metabolism, and function". *Bacteriol Rev*. 30 (4): 772–94.
- 5) Terkeltaub RA (July 2001). "Inorganic pyrophosphate generation and disposition in pathophysiology". *Am. J. Physiol., Cell Physiol*. 281 (1): C1–C11.
- 6) Jeong, Sang-Ho, *et al.* "Pyrophosphatase overexpression is associated with cell migration, invasion, and poor prognosis in gastric cancer." *Tumor Biology* 33, no. 6 (2012): 1889-1898.
- 7) Baykov, A.A., *et al.* 1999. Cytoplasmic inorganic pyrophosphatase. *Prog. Mol. Subcel. Biol*. 23, 127–150
- 8) Tsuji, A; *et al.* "The effects of temperature and pH on the growth of eight enteric and nine glucose non-fermenting species of gram-negative rods." *Microbiol Immunol*. 1982. 26(1). p. 15-24.
- 9) Campos-Acevedo, A.A. *et al.* "First attempts to crystallize a non-homogenous sample of thioredoxin from *Litopenaeus vannamei*: What to do when you have diffraction data of a protein that is not the target?" *Biochem Biophys Rep* (2017).
- 10) UNIPORT accession number: A0A080T5H9. Mar 2017.
- 11) Salminen, T *et al.* "An unusual route to thermostability disclosed by the comparison of *thermus thermophilus* and *escherichia coli* inorganic pyrophosphatases." *Protein Science*. 1996. 5. p. 1014-25. Doi:10.1002/pro.5560050604
- 12) Lahti, R. *et al.* "Cloning and characterization of the gene encoding inorganic pyrophosphatase of *Escherichia coli* K-12." *J. Bacteriol*. 1988. 170. p. 5901-07
- 13) Ristucci, P. and Burke, C. "Infection Control" *Cambridge University Press on behalf of The Society for Healthcare Epidemiology of America*. p. 343-8
- 14) Tris-HCL Product Information. Cell Culture. Product Code: TC073.
- 15) Good Buffers (Biological Buffers). Interchim. Uptima.

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