Steps Toward the Inhibition of Aminoglycoside 6'-*N*-acetyltransferase Type Ib

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Introduction

Aminoglycoside 6'-*N*-acetyltransferase type Ib has been linked to the development of antibiotic resistance in Gram-negative pathogens.

The gene encoding the cr-variant is dominant in over 50% of human gut microbiota genomes (Kim, 2018).

Vetting (2008) shows that AAC(6')-Ib catalyzes the acetylation of both aminoglycosides and fluoroquinolones.

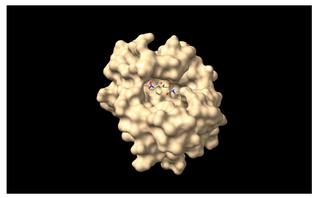
Aggregation of AAC(6')-Ib correlates with higher levels of resistance to amikacin until the relationship plateaus (d'Acoz et al., 2023).

We seek to gain information regarding AAC(6')-Ib to develop inhibitors that will enable antibiotics such as amikacin to kill Gram-negative pathogens that would otherwise be difficult and expensive to treat (Rocha et al., 2007).

Aim

The goal of our research is to decrease the threat to public health that antibiotic resistance employs due to bacterial plasmids containing AAC(6')-Ib coding sequences.

We will accomplish this task by analyzing the protein and finding the molecules with the best molecular docking scores to design inhibitors of the enzyme.

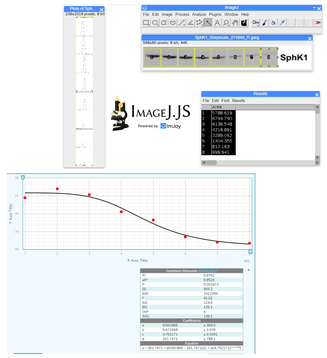


This image provides an example of molecular docking which is a primary focus of this presentation.

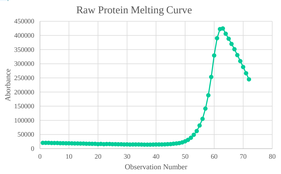
Methods

Steps toward designing AAC(6')-Ib inhibitors:

- ❑ Created media to harbor *E coli*
- ❑ Used *E coli.* vectors for growing AAC(6')-Ib
- ❑ Centrifuged protein and collected concentrate
- ❑ Purified the protein using methods indicated below
- ❑ Assessed enzyme kinetics using Excel
- ❑ Analyzed thermal activity using Excel
- ❑ Performed molecular docking in ChimeraX to find the best-fitting inhibitors that can be synthesized



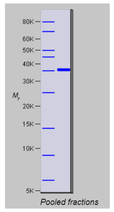
Cellular Thermal Shift Assay and sigmoidal fit with equation. Done using ImageJ software and curve fit software.



By finding the second derivative of this raw data and calculating where the second derivative crosses the x-axis we were able to determine the melting point of the protein.

Purification of protein 4 from Default Mixture

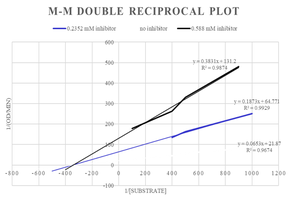
Method	Protein (mg)	Enzyme (Units)	Yield (%)	Enrich	Cost (h/100U)
Initial	511.0	7000.0	100.0	1.0	0
Ion exchange	244.4	6999.8	100.0	2.1	0.100
Gel filtration	97.1	6999.3	100.0	5.3	0.314
Affinity chromatography	10.5	2099.7	30.0	14.6	2.715



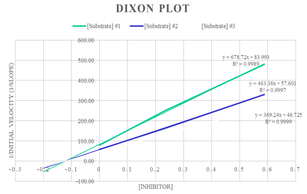
This is the series of techniques we used to purify our enzyme to get one clear band in our 1D PAGE gel.

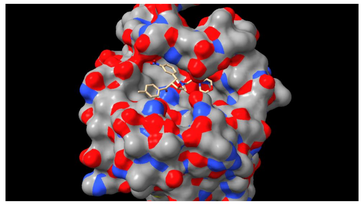
Results

- ❑ Determined a sigmoidal line of best fit to better understand the cellular thermal shift
- ❑ Calculated the melting point of our protein to be 59.42
- ❑ Performed ion exchange column, gel filtration, and affinity chromatography to purify our protein
- ❑ Used Michaelis-Menten kinetics to determine shifts in the Km and Vmax values as we increased inhibitor and substrate concentrations
- ❑ Determined the inhibitor works through non-competitive inhibition
- ❑ Used Dixon plot analysis and determined the Ki value to be 0.1249
- ❑ Found ZIN000008818057 to be the best docking molecule and determined its optimal position at a docking score of -9.6



M-M Double Reciprocal Plots and Dixon Plots were used to determine the Km, Vmax, and Ki for our enzyme.





This 3D model shows a depiction of our best docking molecule in its optimal position. This analysis was done using ChimeraX.. This produced a docking score of -9.6.

Conclusion

We have purified AAC(6')-Ib and calculated its melting point, Km, Vmax, and Ki, and have found a molecule that performs at a docking score of -9.6.

Now we plan to move forward in designing and producing an inhibitor that has properties like ZIN000008818057 and works to inhibit AAC(6')-Ib. An inhibitor will enable antibiotics such as amikacin to kill Gram-negative pathogens that would otherwise be difficult and expensive to treat (Rocha et al., 2007).

Once we can design an effective inhibitor, we will need to do drug trial runs in mice and eventually clinical trial runs in humans to get FDA approval for inhibitor drug use.

This research has the potential to lead to a reduction in unresponsive antibiotic use, increased public health, and decreased healthcare costs.

References

•d'Acoz, O. d'Udekem, Hue, F., Ye, T., Wang, L., Leroux, M., Rajngewerc, L., Ramirez, M. S., Reisner, W., Tolmasky, M. E., & Reyes-Lamothe, R. (2023). *Dynamics and quantitative contribution of the aminoglycoside 6′- N -acetyltransferase type Ib [AAC(6′)-Ib] to amikacin resistance* [Preprint]. Microbiology. https://doi.org/10.1101/2023.09.05.556435

• Kim, Choon & Villegas-Estrada, Adriel & Hesek, Dusan & Mobashery, Shahriar. (2007). Mechanistic Characterization of the Bifunctional Aminoglycoside-Modifying Enzyme AAC(3)-Ib/AAC(6′)-Ib‘ from Pseudomonas aeruginosa. Biochemistry. 46. 5270-82. 10.1021/bi700111z.

• Kim, D.-W., Thawng, C. N., Lee, K., & Cha, C.-J. (2018). Revisiting polymorphic diversity of aminoglycoside n-acetyltransferase aac(6′)-ib based on bacterial genomes of human, animal, and environmental origins. *Frontiers in Microbiology*, *9*, 1831. https://doi.org/10.3389/fmicb.2018.01831

•Rocha, K., Magallon, J., Reeves, C., Phan, K., Vu, P., Oakley-Havens, C. L., Kwan, S., Ramirez, M. S., LaVoi, T., Donow, H., Chapagain, P., Santos, R., Pinilla, C., Giulianotti, M. A., & Tolmasky, M. E. (2021). Inhibition of aminoglycoside 6′-n-acetyltransferase type ib (AAC(6′)-Ib): Structure–activity relationship of substituted pyrrolidine pentamine derivatives as inhibitors. *Biomedicines*, *9*(9), 1218. https://doi.org/10.3390/biomedicines9091218

•Vetting, M. W., Park, C. H., Hegde, S. S., Jacoby, G. A., Hooper, D. C., & Blanchard, J. S. (2008). Mechanistic and structural analysis of aminoglycoside N-acetyltransferase AAC(6′)-Ib and its bifunctional, fluoroquinolone-active AAC(6′)-Ib-cr variant. *Biochemistry*, *47*(37), 9825–9835. https://doi.org/10.1021/bi800664x

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