Introduction

Antibiotic resistance is a problem of utmost importance in the medical community. Because there have been very few breakthroughs toward new antibiotics in recent years, it is critical to explore new ways to inhibit bacterial proliferation and growth. The fact that many antibiotics operate on similar control mechanisms only intensifies the urgency to develop antibiotics with new mechanisms of inhibition to avoid resistance. There are several intrinsic differences between prokaryotic (bacterial) and eukaryotic (animal) cells. Unfortunately, the large majority of current antibiotics all take advantage of ribosomal differences which means some bacteria have evolved new ribosomal techniques to avoid being killed by several current antibiotics, thus the rise of antibiotic resistance. N-Succinyl-L,L-diaminopimelic acid desuccinylase enzyme (DapE) is an enzyme in the late stage of the meso-diaminopimelic/lysine biosynthetic pathway (Figure 1). DapE is a dimetalloenzyme that catalyzes the substrate N-succinyl-L,L-diaminopimelic acid (LL-SDAP) to succinate and diaminopimelinate (DAP) (Scheme 1). DapE activity results in production of meso-diaminopimelinate (mDAP) from DAP which is ultimately converted to lysine. Lysine and mDAP are both essential components for peptidoglycan cell wall synthesis. This succinate enzymatic pathway is found in all Gram-negative and most Gram-positive bacteria. Because of this, DapE has been identified as an attractive bacterial target.

Assay Protocol

Buffer
The assay is run in 50 mM HEPES buffer at pH 7.5. This buffer tops off each reaction vial at 200 μL. Variable amounts per reaction are due to variable volumes of reagents added. All reactions are also run with 5% DMSO in solution due to solubility of some inhibitors.

Enzyme
We use the DapE enzyme isolated from Haemophilus influenzae and it is run at roughly 9 mM in the assay. Due to the delity of the active enzyme, mixing must be done via repetitive uptake and dispensing of solution gently in the pipette to avoid denaturing the enzyme.

Substrate
The substrate is an N-methylated SDAP derivative (N-methyl-LL-SDAP) seen in Scheme 2.1 and it is run at 2 mM concentration in the assay.

Inhibitors
Inhibitors are usually prepared in a stock solution of 100% DMSO and kept at -20°C. They are diluted appropriately in a 50% DMSO/50% buffer solution so that after addition of 20 μL to the 200 μL reaction volume, all tubes will have the 5% DMSO solution mentioned above. This does help with the solubility of some inhibitors, but DMSO has also been shown to deactivate the enzyme, making it a crucial variable to control.

Spectrophotometric Analysis
When the reaction is complete, nihydin reacts with cleaved substrate turning dark purple and the absorbance is measured at 570 nm. This means the darker wells have high activity and the lighter ones have low activity. Yellow arrow shows high activity and blue shows low activity in Figure 3.

Activity of DapE with DMSO

When looking at our 1.3Å crystal structure (PDB 5uej) with our collaborators, it was observed that there are two sulfates bound in each active site. This prompted us to note that in the substrate bound structure, two of the carboxylates attached to the substrate are located in the same place. We hypothesized that sulfate also is a competitive inhibitor of the enzyme in addition to our small molecule inhibitors. We then decided to determine the IC50 of lithium sulfate using the same assay outlined above. The results and logarithmic inhibition curve are displayed below in Figure 6. The IC50 for sulfate was determined to be 31 ± 13 mM.

Targeted Molecular Dynamics

The results of this prompted our group to begin investigating the distances between these two sulfates as the enzyme goes through closure. We began this by investigating enzymatic conformations in VMD using targeted molecular dynamics (TMD). This involves applying a force to certain atoms in computer software with the intent of reaching a target conformation. As we suspected the two sulfates bound in the active site grow significantly closer as force is applied. This has led us to begin the next leg of our research.

Future Plans

Primarily, we will be focused on obtaining IC50 data for the rest of our library of compounds, including those listed earlier, which will help to direct the synthesis of future compounds. We will also be investigating the inhibition potential of bis-sulfonlates as we predict that the sulfonate groups will bind tightly to the active site, based on the demonstrated inhibition by anionic sulfite. In addition, we will be working with collaborators in molecular dynamics to investigate the activation seen by low concentrations of inhibitors.

Citations


Acknowledgements

Thank you to all of the wonderful mentors especially the graduate students of Dr. Becker’s lab; especially Thahir Habeeb and Dr. Tahirah Heath for their leadership and guidance.

Thank you to all our collaborators, especially Matt Kochert and Dr. Olsen for their insights and contributions in understanding enzyme dynamics.

Finally, a special thanks to Dr. Becker for being an amazing teacher, researcher, and mentor.