Inhibitors of the Bacterial Enzyme ArgE as Potential Novel Antibiotics

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Abstract

Bacteria are becoming increasingly resistant to antibiotics, and therefore there is an urgent need for novel antibiotics targeting alternate pathways. Toward that goal, our research studies inhibitors of the di-zinc metalloenzyme acetylornithine deacetylase (ArgE), an enzyme found in the arginine biosynthesis pathway of bacteria. Because ArgE is only present in bacteria, inhibiting this enzyme would kill bacteria without mechanism-based toxicity to humans. We screened classes of potential inhibitors and tested inhibitors in a 214 nm assay and found IC_{50} values for the most potent compounds. We also tested select inhibitors in a thermal shift assay (TSA) with *Escherichia coli* ArgE to determine the stability of the enzyme in the presence of inhibitors.

Introduction

Due to the prevalence of antibiotic-resistant infections, it is crucial to identify new antibiotics, especially those with a new mechanism of action[1]. An underexplored target for developing such antibiotics is the di-zinc metalloenzyme N^{α}-Acetyl-L-ornithine deacetylase (ArgE).

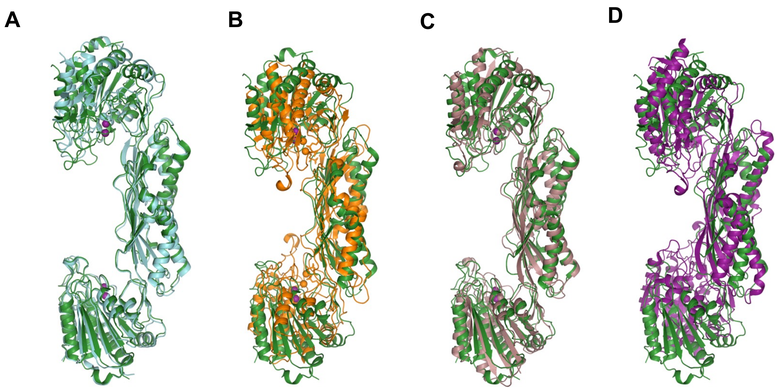


Figure 1[2]: The *E. coli* ArgE dizinc structure (green) compared to similar proteins. (A) *Ec*ArgE mono-zinc form (cyan) (B) *Haemophilus influenzae* DapE (orange) (C) *Enterococcus faecium* DapE (brown) (D) *Rhodopseudomonas palustris* ArgE

The ArgE enzyme is part of the arginine biosynthesis pathway in all Gram-negative and most Gram-positive bacteria[3]. Without this enzyme, the cell is not viable[4]. The enzyme is also similar in structure to DapE, an enzyme our group has worked with previously. Both enzymes have similar homodimeric structures and similar active site residues.

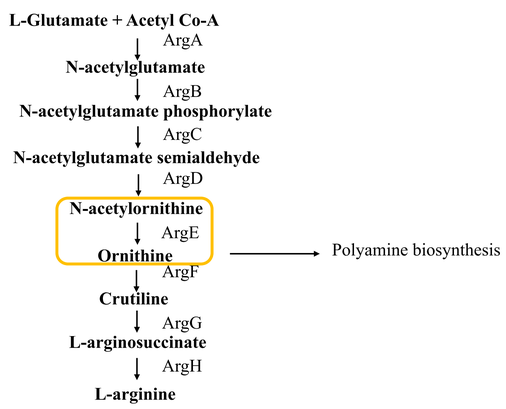


Figure 2: The biosynthetic pathway where ArgE operates.

Work by the Blanchard group found using a 214 nm assay that N^{α}-acetylornithine (NAO) was the substrate for ArgE, forming the products acetate and L-ornithine[3].

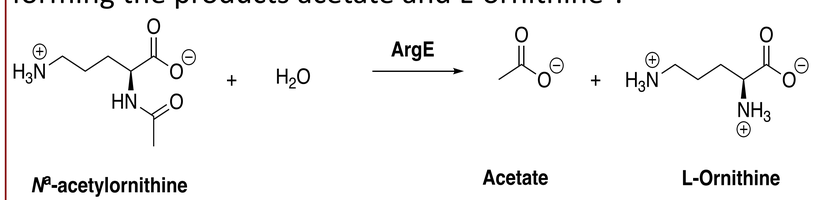


Figure 3: The reaction catalyzed by ArgE, the conversion of N^{α}-acetylornithine (NAO) to acetate and L-ornithine

Methodology

214 nm Assay

To test inhibition of the ArgE enzyme, the activity of the enzyme was measured by monitoring the amount of product created. As the concentration of the product increases, the absorbance of light at 214 nm decreases. This was observed with UV-Vis spectroscopy. The percent inhibition and IC_{50} values were calculated using the absorption data. This 214 nm assay is simple to use and reproducible, but because many organic molecules absorb in the UV region, it is difficult to detect the enzyme's products. This applies especially to druglike molecules.

Ninhydrin Assay

The use of a ninhydrin assay allows detection of more complex inhibitors. Previous work in our group designed a ninhydrin assay with DapE. Given the similarity between DapE and ArgE, we created a ninhydrin assay for ArgE. Ninhydrin reacts with a free amine to form Ruhemann's purple. However, the amine group on NAO would interfere with the ninhydrin assay, so molecules other than the product react, obscuring the results. To address this, we synthesized an analog of NAO, N^5,N^5-dimethyl-ornithine, which substitutes the primary amine with methyl groups, forming a tertiary amine. Ninhydrin reactions with the primary amine product of the substrate analog form Ruhemann's purple.

To validate the assay, its results must be compared to those from the 214 nm assay.

Figure 3: The natural substrate compared to the synthesized analog.

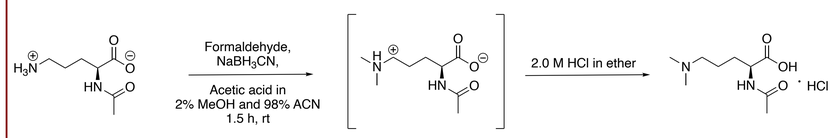


Figure 4[2]: Synthesis of N^5,N^5-dimethyl-N^{α}-acetylornithine

The k_{cat}/K_M and IC_{50} values for the ninhydrin assay are statistically identical to the results from the 214 nm assay, demonstrating the validity of the ninhydrin assay.

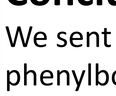
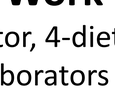
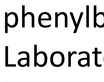
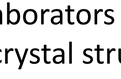
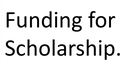
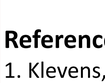
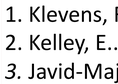
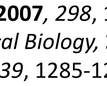
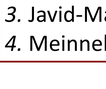
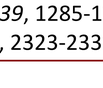
Thermal Shift Assay

To further understand how the inhibitors interact with ArgE, thermal shift assays were run with SYPRO Orange dye, which binds to the enzyme as it denatures, making its fluorescence a marker of the denaturation of the enzyme. The TSA was run with captopril.

Results and Discussion

Fifteen inhibitors were tested in the 214 nm assay with a concentration of 100 μM, with the most potent being 4-diethylamino phenylboronic acid (93.4%±2.2).

Table 1: ArgE Inhibitors

Structure	Name	214 nm assay, NAO: ArgE %inhib at 100 µM	214 nm assay, NAO: ArgE IC50 (µM)	Structure	Name	214 nm assay, NAO: ArgE %inhib at 100 µM	214 nm assay, NAO: ArgE IC50 (µM)
	Captopril	57.8% +/- 0.04	58.7 +/- 5.8		4-hydroxyphenyl boronic acid	18.5% +/- 11.2	
	Phenylboronic acid	46.1% +/- 4.5	132 +/- 10.8		4-(diethylamino)phenyl boronic acid	93.4% +/- 4.6	
	4-Carboxyphenyl boronic acid	78.6% +/- 8.4			4-methylphenyl boronic acid	44.9% +/- 2.2	
	4-Cyanophenyl boronic acid	42.9% +/- 7.1			3,5-bis(trifluoromethyl) phenylboronic acid	0%	
	2-(trifluoromethyl)phenyl boronic acid	41.5% +/- 5.6			4-chlorophenyl boronic acid	67.7% +/- 1.2	
	4-fluorophenyl boronic acid	39.1% +/- 7.8			3-(benzyloxy)phenyl boronic acid	39.7% +/- 2.5	
	N-4-methanesulfonamide phenylboronic acid	37.4% +/- 6.9			(2-methylpropyl) boronic acid	27.4% +/- 4.6	
	4-acetylphenyl boronic acid	32.3% +/- 4.8					

The results for captopril in the 214 nm assay were compared to the results for captopril in the ninhydrin assay and confirmed the validity of the ninhydrin assay. 4-diethylamino phenylboronic acid had the highest potency in the ninhydrin assay as well, as anticipated, with an IC_{50} value of 50.1μM.

The TSA data shows a positive shift in the melting temperature of the enzyme in the presence of inhibitor. This indicates that the enzyme stabilizes when the inhibitor binds.

Table 2[2]: TSA data for EcArgE in the presence of captopril

[captopril]	T_m	K_i
0 µM	56.4 ± 0.24	35.9 ± 5.1 µM
1 µM	56.4 ± 0.17	
10 µM	56.6 ± 0.07	
30 µM	56.6 ± 0.11	
50 µM	57.8 ± 0.30	
70 µM	58.5 ± 0.29	
90 µM	59.1 ± 0.13	
100 µM	60.0 ± 0.35	

Conclusion and Future Work

We sent the most potent inhibitor, 4-diethylamino phenylboronic acid, to our collaborators as Argonne National Laboratory. We hope to find a crystal structure for the inhibitor-bound enzyme.

Acknowledgements

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References

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4. Meinnel, T.; et al. *J. Bacteriol.*, **1992**, 174, 2323-2331.