Substrate Scope Analysis of Biocatalytic Halogenation on Complex Substrates

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Abstract

Malbrancheamide (**Figure 1**) is a fungal natural product with significant vasorelaxation effects and potential as a cardiovascular therapeutic. The dichlorination of the indole ring is key for its biological activity, and this transformation is performed by the flavin dependent halogenase (FDH) MalA (**Figure 2**). This enzyme utilizes a proposed chloramine lysine intermediate to iteratively and selectively chlorinate its natural substrate premalbrancheamide. Halogenases can provide orthogonal selectivity to many chemical methods, making them useful for pharmaceutical applications, while providing selective methods for late-stage functionalization.

Figure 1. Native reaction of MalA

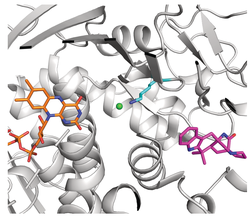

Figure 2. MalA bound to FAD(orange), premalbrancheamide, (pink), Cl^- (green), and lysine (cyan)

This investigation focuses on the substrate scope of the halogenase on complex pharmaceutically relevant substrates in collaboration with the Novartis Institutes for Biomedical Research. The bromination and chlorination reaction conditions were optimized, and the products were structurally characterized by NMR spectroscopy to gain further understanding of the versatility of the wild type enzyme and its mutants.

Background

Due to the importance of halogenation in drug design[1], new methods of selective and efficient halogenation can provide further means of tailoring therapeutics, as many chemical methods lack selectivity. Nature has evolved efficient methods of performing the most chemically challenging reactions under benign catalytic conditions, allowing enzymes to perform these reactions with exquisite site selectivity.

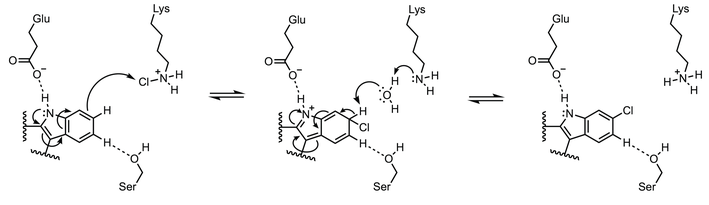

Figure 3. MalA EAS reaction mechanism with chloramine lysine intermediate

MalA is an FDH which halogenates via electrophilic aromatic substitution through a proposed chloramine lysine intermediate[2] (**Figure 3**). This intermediate prevents performing nonselective halogenation through hypohalous acid. Due to the versatility of the enzyme, MalA can halogenate a range of unnatural substrates.

Biocatalysis

Initial high throughput screening was performed at Novartis to investigate MalA's substrate scope. *In vitro* enzymatic reactions were carried out on select non-proprietary compounds which showed activity. These were optimized by varying volume and concentration of halogenase, reductase, substrate, and volume.

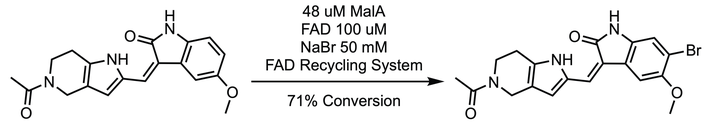

Figure 4. Reaction of Novartis 2-217 under brominating conditions with the wild type MalA to brominate the oxindole C6 position

Reactions were extracted with ethyl acetate, and purified by HPLC. LC/MS was used to confirm halogenation, while NMR was used to determine the location of the halogenation (**Figure 4**).

Mutant Screening

S129A/P85S MalA (**Figure 5**) mutant reactions were performed on compounds sent from the Novartis library. These reactions were screened by LC/MS for halogenation (**Figure 6**).

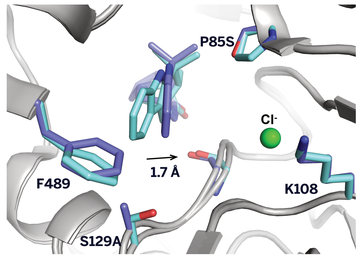

Figure 5. MalA wild-type (teal) and S129A/P85S (navy) overlaid, with premalbrancheamide bound

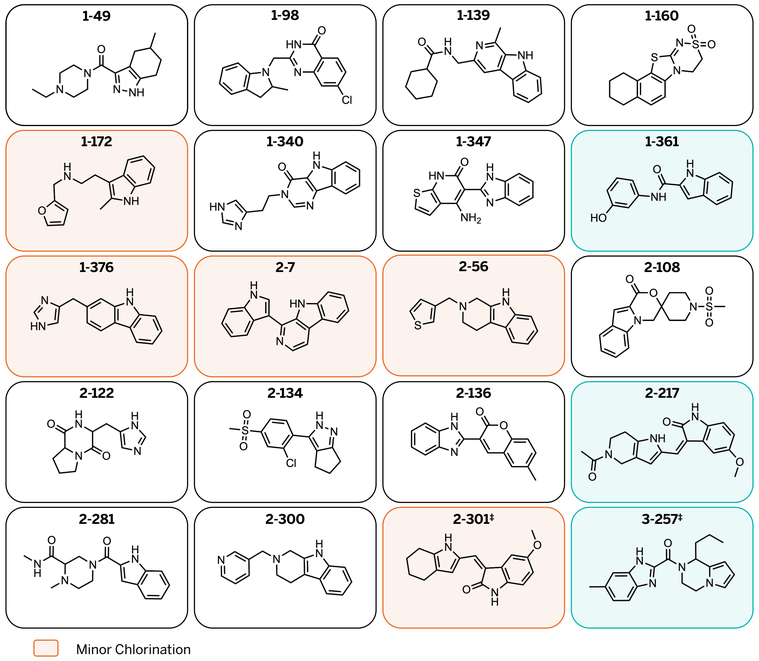

Figure 6. Mutant MalA S129A/P85S screen with Novartis compounds

Conclusions

The flavin dependent halogenase MalA is a versatile enzyme for selective halogenation (**Figure 7**) and late stage C—H functionalization of complex substrates by electrophilic aromatic substitution.

Reactions with unnatural substrates were optimized and scaled up to produce enough material for full characterization. The product of 2-217 (**Figure 4**) was found to be halogenated on C6 of the indole ring.

Screening of the S129A/P85S mutant found six hits out of 20 substrates screened. This shows that the S129A/P85S is useful for halogenation, as well as reactivity on compounds which didn't show halogenation with the wild type enzyme.

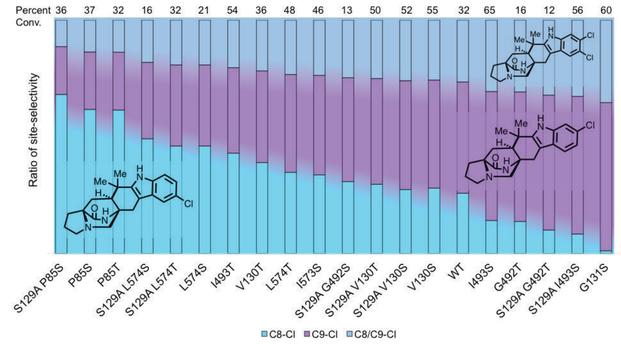

Figure 7. Selectivity of MalA mutants on the natural premalbrancheamide substrate performed by Amy Fraley

References

1. Xu, Z.; Yang, Z.; Liu, Y.; Lu, Y.; Chen, K.; Zhu, W., Halogen Bond: Its Role beyond Drug–Target Binding Affinity for Drug Discovery and Development. Journal of Chemical Information and Modeling 2014, 54 (1), 69-78.
2. Fraley, A. E.; Garcia-Borràs, M.; Tripathi, A.; Khare, D.; Mercado-Marin, E. V.; Tran, H.; Dan, Q.; Webb, G. P.; Watts, K. R.; Crews, P.; Sarpong, R.; Williams, R. M.; Smith, J. L.; Houk, K. N.; Sherman, D. H., Function and Structure of MalA/MalA′, Iterative Halogenases for Late-Stage C–H Functionalization of Indole Alkaloids. *Journal of the American Chemical Society* **2017**, *139* (34), 12060-12068.

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