MalA: an iterative halogenase for late-stage C-H functionalization of indole alkaloids

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The abundance of halogenated natural products has led to a great advancement in characterizing biosynthetic halogenation strategies. Of the various classes of halogenating enzymes, flavin-dependent halogenases (FDHs) have been widely studied. These FDHs are of particular interest due to their utility in halogenating the idole moiety, which is commonly found in pharmaceutically relevant molecules. The ability to selectively halogenate C-H bonds in highly complex molecules through synthetic methods has posed a formidable challenge due to the abundance of chemically equivalent C-H bonds that are difficult to activate. With the vast majority of biologically active natural products undergoing late-stage functionalization by tailoring enzymes, there is a unique opportunity to leverage the power of halogenating enzymes to perform difficult chemical transformations. Early efforts to modulate the selectivity of halogenases achieved shifts in regioselectivity and slight modifications to substrate scope, while recent efforts have succeeded in engineering them for a broad range of regioselectivities and accepted substrates. Through this work, we identified the versatile halogenase involved in malbrancheamide biosynthesis and demonstrated its potential as a biocatalyst for late-stage halogenation.

Malbrancheamide is a complex dichlorinated indole alkaloid produced by the ascomycete *Malbranchea aurantiaca.* The discovery of malbrancheamide was initiated by a search for calmodulin antagonists, and several thorough studies have characterized its significant vasorelaxant effect. Since the chlorination of the indole ring differentiates this molecule from the rest of its class and significantly contributes to the biological activity, we were motivated to delve into the mechanism of its iterative halogenation by the FDH MalA. MalA was found to catalyze both chlorination and bromination reactions of hexacyclic indole alkaloids, and used to chemoenzymatically generate a library of halogenated indole alkaloids. Michaelis-Menten model kinetics displayed a lack of selectivity between the two sites of halogenation, but also a higher catalytic efficiency for the second chlorination reaction. Additionally, X-ray co-crystal structures of MalA with the natural substrate premalbrancheamide, and the monochlorinated malbrancheamide B and isomalbrancheamide B, revealed the mode of binding of the substrates within the active site. The structural data also led to a new mechanistic proposal for the electrophilic aromatic substitution catalyzed by MalA. With this knowledge in hand, future work involves engineering MalA as a biocatalyst for late-stage halogenation of complex substrates facilitated by computation studies and directed evolution.