Halogenase Engineering for the Generation of Novel Natural Products

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Abstract: Halogenases catalyze the incorporation of halogen atoms into organic molecules. Given the importance that halogenation has on the biological activity of small molecules, these enzymes have been subjected to intense engineering efforts to make them more suitable for biotechnology applications. The ability to biohalogenate complex molecules provides, in principle, the opportunity to quickly generate a series of analogs with novel or improved properties. Here we discuss the potential and limitations of using halogenases as biocatalysts, including recent advances to engineer halogenases to generate halogenated natural product analogs.
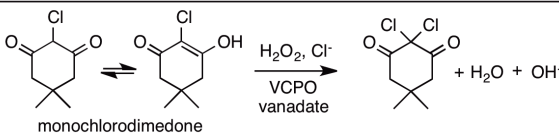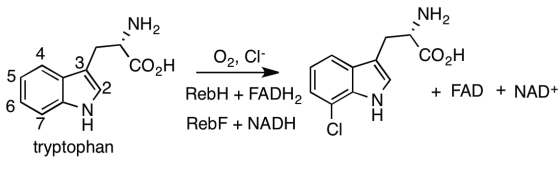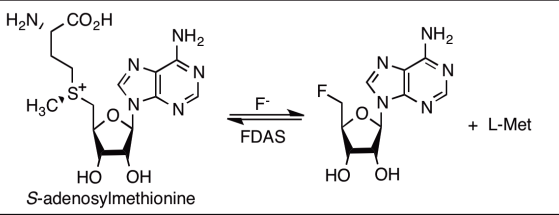
Introduction

Halogen substituents (F, Cl, Br and, to a lesser extent, I) are a chemical functionality frequently used to generate compounds with novel or improved activities and enhanced pharmacological efficacy. In addition to modulating compound activity and physical properties, chlorine and bromine substituents can also act as handles for further selective chemical modification.[1] Nearly one-quarter of pharmaceuticals and agrochemicals currently in use are halogenated.[2] Halogenated organic compounds now pervade industrial, agrochemical, pharmaceutical and material products and are important synthetic building blocks in metal-catalyzed carbon coupling reactions used for the production of organic fine chemicals.[3] Though halogenated compounds are widely used in organic synthesis, the chemical process of halogenating complex molecules remains challenging. Conventional halogenation chemistry is often a noxious, environmentally hazardous process that requires harsh reaction conditions involving toxic reagents and solvents. Additionally, these reactions can suffer from poor regioselectivity resulting in more than one regioisomer and unwanted byproducts that require separation and careful disposal due to their toxicity and persistence in the environment.[4] Enzyme catalysis has emerged as a complement to organic synthesis, since enzymatic reactions, which occur in water under mild conditions, are an environmentally friendly, green approach to carry out complex chemistry.[3b] Additionally, enzymatically catalyzed reactions proceed at high rates with high regio- and stereo- selectivity, eliminating the need for protecting or activating groups, thereby leading to more efficient synthesis routes.[3b] The recent discovery of halogenases makes enzymatic halogenation possible.

Living organisms are known to produce over 4700 halogenated natural products.[1] Halogenated natural products most commonly contain chlorine or bromine, with relatively few iodine or fluorine containing metabolites having been identified to date.[1] The enzymes that catalyze chlorination, bromination and iodination can be broadly characterized into two groups: the highly substrate specific halogenases requiring dioxygen and the promiscuous haloperoxidases utilizing hydrogen peroxide (Figure 1).[5] A haloperoxidase was the first discovered halogenase, reported over 50 years ago.[6] These halogenases generate hypohalous acid, which diffuses out of the active site to react with an organic substrate. Since the mechanism of the reaction entails the use of a highly reactive agent outside of the controlled environment of the enzyme active site, haloperoxidases typically lack substrate specificity and regioselectivity, resulting in the generation of a range of mono- di- and tri- halogenated products.[1] The first regioselective halogenating enzyme discovered was the tryptophan 7 halogenase PrnA involved in pyrrolnitrin biosynthesis (Figure 1).[7] There are now a number of characterized substrate specific halogenases from a variety of organisms that utilize oxidative enzymatic mechanisms requiring iron or flavin cofactors. In contrast to chlorination, bromination and iodination, fluorination has only one characterized enzyme to date. This fluorinase is FDAS (5'-fluoro-5'-deoxyadenosine synthase) from *Streptomyces cattleya*, which uses a nucelophilic mechanism (Figure 1).[1] For more information on halogenase classes and mechanisms the reader is directed to a number of excellent recent reviews (Figure 1). [1, 8]

Due to the challenging and toxic nature of many conventional chemical halogenation reactions, advancements in understanding enzymatic halogenation have given rise to probing the biotechnological potential of these enzymes. Halogenases function at low temperature, neutral pH, and utilize benign halide salts, thereby avoiding toxic halogenating agents used in much halogenation chemistry.[3b] Moreover, enzymes facilitate halogenation at specific substrate sites not accessible by conventional chemistry methods. The haloperoxidases have been considered for biotechnological applications, but their lack of product regioselectivity has led to unwanted by-products, as with non-enzymatic halogenation chemistry.[1, 9] Thus, most efforts to apply biocatalytic halogenation have focused on advancing the potential of regioselective halogenases. Though promising, the utility of halogenase biocatalysts is hindered by enzyme instability, poor catalytic efficiency, and limited substrate range.[3b, 10] Here, we provide an overview of engineering efforts to improve the biotechnological prospects of these enzymes and highlight efforts to incorporate halogenases into natural product pathways to generate complex natural product analogs that would be difficult to access by traditional synthetic methods.

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Halogenase Type	Example Reaction	Enzymes discussed in text
Haloperoxidase accepts I⁻, Br⁻, Cl⁻		VCPO (vanadium dependent chlorinase) CPO (chloroperoxidase) BPO-1A (non-heme bromoperoxidase)
Flavin-dependent halogenase accepts Br⁻ and Cl⁻ the required FADH$_2$ is provided by a partner-protein reductase		RebH (7-tryptophan halogenase) PrnA (7-tryptophan halogenase) ThaI (6-tryptophan halogenase) PyrH (5-tryptophan halogenase) Rdc2
Fluorinase accepts F⁻, Cl⁻		FDAS SalL is a chlorinase that catalyzes the homologous reaction using Cl⁻
Iron(II) α-ketoglutarate halogenase (also called non-heme iron dependent)		SyrB2

Figure 1. Schematic illustrating the various halogenases discussed in the text. A representative reaction for each halogenase class is shown in the central column.

Enhancing stability and catalytic efficiency

Ideally, enzymes used as biocatalysts for academic synthesis or industrial applications should be highly selective, stable, and catalytically efficient. While halogenases have been studied extensively on an analytical scale, preparative-scale enzymatic halogenation still requires optimization.[3b] Halogenation on a large scale is inefficient predominately because halogenases are unstable *in vitro*, with total turnover numbers less than 200. Furthermore, halogenases often suffer from low catalytic efficiency, with many halogenases showing k_{cat} turnover rates on the order of 1 min⁻¹.[3b] These rates can further decrease in the presence of the high substrate concentrations that are required for biotechnological applications.[3b]

Immobilization of haloperoxidases
Enzyme immobilization onto a solid support represents a promising way to perform large-scale halogenation reactions *in vitro* as immobilization can improve stability and can allow capture and recycling of the biocatalyst.[11] Chloroperoxidase (CPO) from *Caldariomyces fumago* is a commercially available heme enzyme that catalyzes several different reaction types in addition to halogenation such as sulfoxidation, hydroxylation, and epoxidation.[11] However, like many halogenases, its use as a biocatalyst is hindered by poor stability at high temperatures and in the presence of high concentrations of its substrate, hydrogen peroxide.[11] Hydrogen peroxide oxidizes the heme

group of the protein, leading to irreversible enzyme deactivation.[12] However, several groups have successfully reported enhancing CPO activity by immobilization.[11] In one recent example, De Matteis and co-authors explored CPO chlorination efficiency in response to immobilization in various polysaccharide-silica matrices. CPO enzyme entrapped in a silica gel doped with chitosan polysaccharide greatly increased biocatalyst reusability. Under optimal conditions CPO could be reused five times without loss of substrate conversion ability and could be used up to 18 times in total. The entrapped enzyme also showed enhanced thermal stability compared to the free enzyme. After two hours at 70°C the immobilized enzyme retained over 95% of its chlorination activity whereas the free enzyme had no activity under these conditions.[11]

Immobilization of flavin-dependent halogenases
RebH, a 7-tryptophan halogenase from *Lechevalieria aerocolonigenes,* is a flavin-containing enzyme that catalyzes the regioselective chlorination or bromination of tryptophan and closely related analogs, but product yields are generally limited by RebH instability.[10, 13] Frese et. al. used a solid support strategy to generate halogenated tryptophan on a gram scale.[3b] Unlike CPO, which is commercially available as a purified enzyme, RebH requires overexpression and purification from a heterologous host. To simplify isolation of the protein, Frese used a CLEA (cross-linked enzyme aggregates) strategy to combine crude fractionation and immobilization into one-step such that crude cell extract could be used.[3b] To allow for continous cofactor regeneration, flavin reductase and alcohol
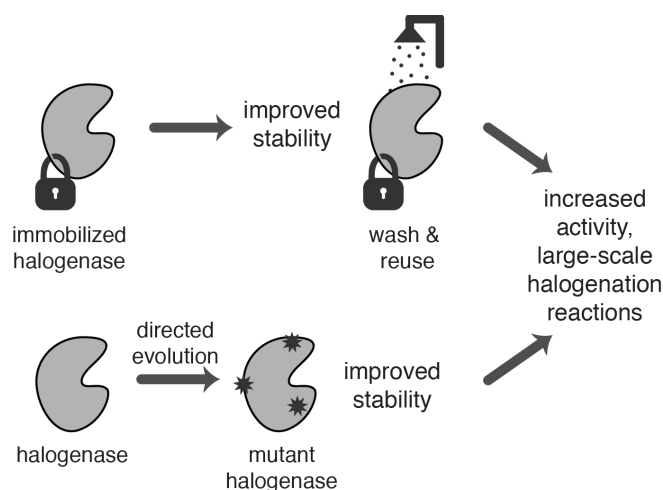
Figure 2. Halogenases are often unstable *in vitro* and have low catalytic efficiency. Enzyme immobilization and directed evolution have been used to increase enzyme stability and activity allowing for halogenation under more rigorous conditions and for large scale preparation.

dehydrogenase proteins were added to an *E. coli* lysate containing overexpressed RebH. The mixture was precipitated with ammonium sulfate and crosslinked using glutaraldehyde to generate a solid multifunctional cross-linked enzyme biocatalyst. Under optimal conditions 99% of all three proteins were simultaneously immobilized, though the activity of RebH dropped to 30% compared to the free enzyme.[3b] Despite the reduction in RebH activity, this immobilized protein system was used on a preparative scale to catalyze the conversion of 1 g of tryptophan into 7-bromotryptophan in 8 days. These CLEAs can be reused at least ten times and better retain activity when stored at 4°C compared to free enzyme.[3b]

Work on CPO and RebH demonstrate that halogenase immobilization can lead to increased product formation, primarily by enhancing long-term enzyme stability. Additionally, because the solid supports are washable and easily separated from reaction components, product recovery is simplified and the halogenase biocatalysts can be re-used.[11] Biocatalysts with both thermal stability as well as the capacity to function in organic solvents could be beneficial to large-scale practical applications that require improved substrate solubility at higher temperatures or in organic solvent, longer catalyst lifetimes, or reactions performed at higher temperatures to increase reaction rates.[10, 14]

Directed evolution of a haloperoxidase
Mutagenesis can also enhance enzyme efficiency and stability. Directed evolution is one approach to generate enzymes with changed function and properties and does not require knowledge of the three-dimensional structure of the enzyme.[10, 14] [14]Ron Wever's laboratory reported the earliest example of directed evolution of a haloperoxidase.[12] The native vanadium chloroperoxidase (VCPO) from *Curvularia inaequalis* is most active at mildly acidic pH values (pH 5), but possible

biotechnological applications such as generating biocides for antifouling applications in seawater would require activity at slightly alkaline pH values. Hasan et. al. used directed evolution to generate a VCPO triple mutant, P395D/L241V/T343A, that has a bromination turnover rate 100 fold higher at pH 8 than native VCPO.[12] This triple mutant also has increased brominating activity (6 fold) and chlorinating activity (2 fold) at pH 5, indicating that this directed evolution study successfully increased the catalytic efficiency of a haloperoxidase.[12] These mutations are believed to cause a change in the electron density on the oxygens of the vanadate cofactor and alter the electrostatic environment near the halide-binding residue Phe-397. In another recent example, Yamada and co-workers used one round of directed evolution on a non-heme (metal-free) haloperoxidase from *Streptomyces aureofaciens* that uses a catalytic mechanism similar to a perhydrolase. This generated an enzyme with improved thermostability and stability in organic solvents.[14] The identified BPO-A1 mutant contains two amino acid point mutations and has a slight improvement in catalytic halogenation efficiency compared to the native enzyme. The authors hypothesized that the improvements are a result of stabilization of an alpha-helix in the protein, changes in surface accessible amino acid residues and enhancement of the interaction between subunits.[14]

Directed evolution of a flavin-dependent halogenase
Poor and co-workers reached similar conclusions regarding the increased thermal stability of mutant RebH enzymes that they generated through three rounds of directed evolution.[10] One mutant, 3-LR, produced 100% more 7-chlorotryptophan than wild-type enzyme at their respective optimal temperatures, which for 3-LR is 5°C higher than wild-type. Another generated mutant, 3-LSR, remained active at elevated temperatures for three times longer than wild-type RebH. Though selecting for mutants with improved thermal stability, the generated mutants also showed improved conversions for the unnatural substrates 2-aminonapthalene, tryptoline, 2-methyltryptamine.[10] In accordance with Yamada's hypotheses, Poor reasoned that the eight amino acid changes of 3-LSR lead to increased thermal stability by increasing protein rigidity, enhancing packing with neighbor residues and changing surface accessible amino acids.[10]

In summary, both mutagenesis and protein immobilization have produced halogenases with increased stability, thereby allowing the possibility of preparative scale chemoenzymatic synthesis of halogenated compounds. To the best of our knowledge, there have been no reports of engineered halogenase mutants immobilized onto solid supports. It would be interesting to determine if immobilizing evolved halogenases results in an additive effect on protein stability and product yields.

Engineering substrate specificity and exploiting substrate promiscuity

Unfortunately, efficient and selective routes to organohalogens continue to be hindered by the narrow substrate range and product profiles of halogenases. Most engineering efforts have focused on changing or increasing the substrate selectivity and regioselectivity of the organic small-molecule substrate. However, recent work demonstrates that the specificity for the halide co-substrate can be modulated by making a single amino acid mutation to the active site. The resulting change in affinity for the halide group results in the switch of a brominating enzyme into a chlorinating enzyme.[15]

Swapping nucleophilic fluroinases and chlorinases

Engineering of halogenases to display increased promiscuity for the organic small-molecule substrate would be of great benefit to synthetic applications and downstream generation of high-value halogenated compounds. Reports have demonstrated that some regioselective halogenases are able to accept a limited range of substrate analogs that closely resemble the natural substrate, albeit with reduced catalytic efficiency.[13, 16] This promiscuity has led to the generation of halogenated natural product analogs. The one known fluorinase (FDAS), as well as one recently discovered chlorinase isolated from *Salinispora tropica* (SalL), catalyzes the nucleophilic attack of chloride or fluoride at C5' of the ribosyl moiety of SAM to produce L-Met and 5'-chloro- or 5'-fluoro- 5'-deoxyadenosine (CIDA or FDA). Thomsen and co-authors used SalL, the chlorinase, and FDAS, the fluorinase, to generate S-adenosylmethionine (SAM) analogs.[16c] Enzymatic diastereoselective syntheses of SAM analogs are highly useful because chemical synthesis of these compounds suffers from low yields and separation of the resulting diastereomers is challenging. *In vitro* equilibrium favors the formation of SAM from CIDA and L-Met, and Thomsen et. al. took advantage of this equilibrium to generate SAM analogs.[16c] When incubated with L-Met substrates and CIDA, the enzymes FDAS and SalL catalyze the formation of a variety of SAM analogs. A series of FDAS and SalL point mutants centered around the active site were also tested for their ability to generate SAM analogs, but unfortunately none of the tested mutants conclusively demonstrated altered substrate selectivity compared to wild-type enzyme.[16c]

Exploiting the substrate promiscuity of a fluorinase

FDAS also has been used to generate [18F]fluoride labeled molecules for positron emission tomography (PET).[17] PET is rapidly expanding into clinical use for imaging various biological processes. Enzymatic synthesis of [18F]fluoride labeled molecules is advantageous over current chemical processes because it eliminates the need to secure dry [18F]fluoride, since enzymatic labeling is performed in aqueous solution.[17] When FDA is bound in the FDAS active site, the C-2 position of adenine is close to the enzyme surface.[17b] The O'Hagan group demonstrated that an acetylene substituent at this C-2 position extends outward into the solvent. The acetylene was extended with a polyethylene glycol linker and an RGD (arginylglycylaspartic acid) peptide, which is used for PET tumor imaging, was attached. Despite the changes to the structure, FDAS is able to catalyze the fluorination of this

substrate presumably because the C-2 moiety of adenine points out of the enzyme into the solvent.[17b] The ability of FDAS to use [18F]fluoride as a nucleophile allowed the creation of labeled PET radiotracers that successfully allowed for *in vivo* imaging in animal models.[17] The C-2 position of adenine can be exploited to generate additional PET radiotracers with other linkers and peptides.[17b]

Exploiting the substrate promiscuity of a flavin-dependent halogenase

Several groups have addressed the issue of substrate tolerance within the family of regioselective flavin-dependent tryptophan halogenases. Wild-type RebH and PrnA, 7-tryptophan halogenases, and PyrH, a 5-tryptophan halogenase, can all accept tryptophan substrate analogs.[13, 16a, 16b] For many substrates the halogenases continue to catalyze regiospecific reactions, though the resulting regioselectivity can differ from the natural substrate. However, owing to active site orientation, certain substrate analogs are generated as a mixture of regioselective products.[13, 16a, 16b] Recent progress has been made to engineer these tryptophan halogenases to utilize larger substrates with increased catalytic efficiency and customizable regioselectivity.

Susbstrates accepted by wild-type RebH and various RebH mutants
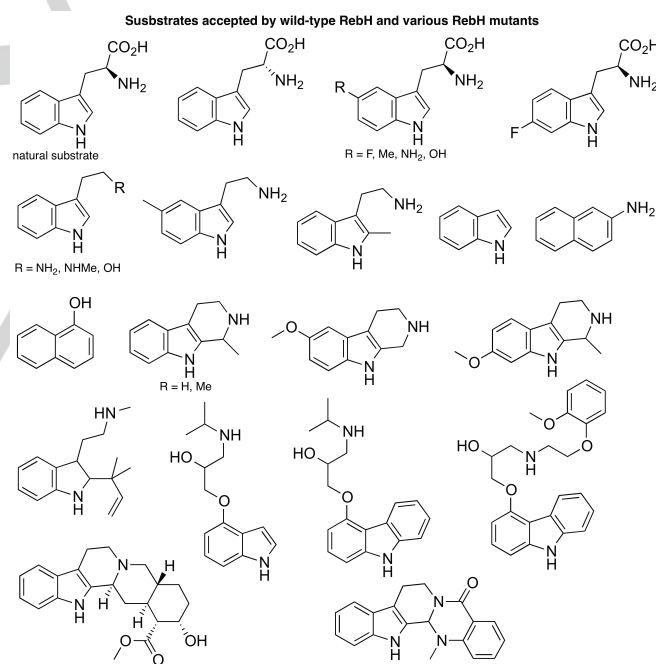
Figure 3. Through rational-design and directed evolution researchers have generated halogenases with increased catalytic efficiency toward a variety of substrates. Here, as an example, we illustrate the substrate tolerance of RebH mutants. RebH can accept Cl- and Br- anions leading to the creation of a variety of halogenated products.

Mutation of flavin-dependent halogenases to broaden substrate specificity and product regioselectivity

Directed evolution was used to generate RebH mutants that can tolerate substrates substantially larger than the natural substrate

tryptophan.[2] A RebH variant (3-SS) has increased k_{cat} and decreased K_m values, resulting in a catalytic efficiency that is 70 times higher than wild-type RebH against tryptoline (a substrate structurally similar to tryptophan). Many of the 3-SS mutations are in the active site and it was hypothesized these mutations affect binding of the substrate. Further evolution by random mutagenesis generated a variant, 4-V, that is able to catalyze chlorination of substrates not recognized by wild-type RebH. These substrates contain substituents on multiple positions of the indole ring as well as much larger natural product substrates such as yohimbine, a naturally occurring alkaloid, and pindolol, a synthetic beta blocker.[2] The 4-V variant contains a mutation far removed from the active site of the enzyme and thus it is unclear why this enzyme can accommodate larger substrates.[2] Distal mutations, which can greatly affect catalysis, are challenging to discover by rational approaches, pointing to the power of directed evolution for development of useful new enzymes. Nevertheless, a structure-guided approach successfully identified a RebH Y455W mutant that maintains regioselectivity to the 7-position of the indole, but preferentially chlorinates tryptamine over tryptophan.[18] Glenn and co-authors hypothesized that replacement of tyrosine 455 with the bulkier tryptophan allows favored access of the smaller tryptamine over the larger tryptophan substrate to the enzyme active site.[18]

The ability to manipulate the regioselectivity of enzymatic halogenation among a wide range of small-molecule substrates would be of considerable benefit to the preparation of halogenated complex molecules. A point mutation in the 5-tryptophan halogenase PrnA (F103A) causes the enzyme to produce 5-halotryptophan in addition to the natural product 7-halotryptophan, demonstrating that enzyme engineering can modulate the regioselective product that is generated.[7] It is hypothesized that this mutation alters the substrate orientation in the active site allowing for halogenation in both the 5- and 7-positions of the indole ring.[7] Similarly, Sheperd et. al. found selected mutations in the PrnA active site both increase the turnover number for the unnatural substrate anthranilic acid compared to wild-type PrnA and affect the ratio of 5- to 3-chloroanthranilic acid that is produced. Crystal structures of the mutant PrnA suggest that, again, substrate positioning in the rearranged active site plays a role in directing chlorination to the ortho or para product.[16b]

Incorporating halogenases into natural product pathways to make new products

There is growing interest in engineering biosynthetic pathways to create complex natural products or natural product analogs that are difficult to synthesize. Halogenated biomolecules have been made using precursor directed and mutasynthesis approaches in which the producing strains are fed chemically synthesized analogs that are incorporated into the native biosynthetic pathways.[19] The increasing number of characterized halogenases now allows a combinatorial biosynthesis approach, in which halogenases are incorporated
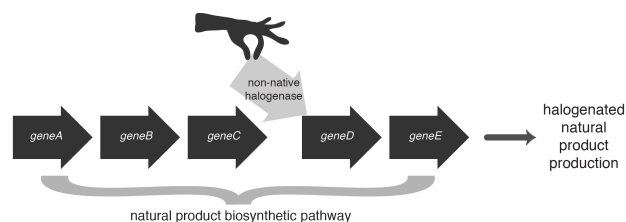
Figure 4. Using a combinatorial biosynthesis approach halogenases can be incorporated into alternative natural product biosynthetic pathways to create halogenated natural products.

into alternative biosynthetic pathways to create novel halogenated products.[20]

Incorporation of flavin-dependent halogenases in metabolic pathways

In an early example, a series of novel halogenated indolocarbazole alkaloid analogs were generated by coexpressing rebeccamycin pathway genes with genes that encode halogenases from different organisms in an actinomycete expression host.[20] By incorporating the non-native PyrH and Thal enzymes, Salas and co-workers created bromo and chloro indolocarbazole derivatives containing halogen moieties at non-natural locations.[20] Similarly, the *prnA* gene was introduced in the pacidamycin producer *Streptomyces coeruleorubidus* to create a strain producing the unnatural compound chloropacidamycin.[21] This chlorine substituent then acted as a handle for site selective cross-coupling to chemically synthesize a variety of derivatives.[21] The O'Connor group reported that incorporation of wild-type RebH and its partner flavin reductase, RebF, into *Catharanthus roseus* root cultures results in production of chlorinated tryptophan derived alkaloids.[22] However, because a downstream enzyme inefficiently catalyzed the decarboxylation of 7-chlorotryptophan to 7-chlorotryptamine, substantial amounts of 7-chlorotryptophan, which has adverse effects on plant health, accumulated.[22] To overcome this issue, the group incorporated RebH Y455W, a rationally designed mutant that preferentially chlorinates tryptamine instead of tryptophan, into *C. roseus* root culture.[18] This example illustrates how engineered halogenases may be better suited than their wild-type counterparts to generate complex natural product analogs.

The late-stage regioselective halogenase Rdc2 from radicicol biosynthesis is postulated to have an open substrate binding site that can accommodate a variety of complex compounds.[16d] Rdc2 is capable of both mono- and di- bromination and chlorination of macrolactones and isoquinolines.[16d, 16e] In combinatorial biosynthesis efforts, Rdc2 was successfully used to chlorinate resveratrol.[23] This substrate promiscuity suggests that Rdc2 may represent a useful starting point to develop and evolve a generic halogenating biocatalyst with broad substrate specificity.

Bottlenecks in metabolic engineering with halogenases

A chloro substituent could be swapped with a fluoro substituent in salinosporamide A biosynthesis. Chromosomal replacement of the chlorinase, SalL, with the related fluorinase, FDAS, in the producer strain resulted in production of fluorosalinosporamide.[24] However, in addition to fluorosalinosporamide, the naturally produced salinosporamide A was also observed, which was attributed to decreased catalytic efficiency of downstream pathway enzymes for the unnatural halogenated biosynthetic intermediate.[24-25]. This problem is often encountered in combinatorial biosynthesis approaches, especially when relying on halogenation of an early stage metabolite.[20] A similar problem was reported in a system based on pyrronitrin biosynthesis. The halogenase ThaI could successfully produce 6-chlorotryptophan in a pyrronitrin producing strain, but since the resulting chlorinated pyrronitrin intermediate was not an accepted substrate by downstream enzymes, the desired halogenated pyrronitrin end product was not obtained.[26] Analogously, RebH was incapable of producing chlorinated cladoniamide and cladoniamide analogs even though these molecules are structurally similar to rebeccamycin, the natural RebH substrate.[27] Development of enzymes that catalyze halogenation of the end product, or engineering of the bottleneck biosynthetic enzymes so that halogenated intermediates are turned over, are needed to address this problem.

Outlook

While enormous strides have been made in making halogenases more practical, numerous challenges remain to be addressed. Fluorine is an important pharmaceutical modification, with 20% - 30% of clinical drugs containing at least one fluorine atom.[28] Consequently, development of effective methods for targeted insertion of fluorine into complex molecules is vital. Recently, it has been demonstrated that fluoroacetate can be used to incorporate fluorine into polyketide products, indicating that metabolic engineering to make fluorinated metabolites is possible.[28] However, a major constraint of fluorinase based technology is the high substrate specificity of the FDAS enzyme. Structural analysis has aided the identification of substrates that are tolerated by FDAS, but to date engineering of this enzyme to increase substrate scope has proved challenging.[17b] Additionally, FDAS remains the only discovered fluorinase; the discovery of naturally occurring flourinases with different substrate specificities would greatly enhance engineering efforts to widen the technological potential of this enzyme class.

The iron(II) α-ketoglutarate halogenases were not discussed in this highlight since little engineering work has been done on these enzymes. These enzymes typically halogenate aliphatic carbons, which can be a difficult step in a synthetic chemical route.[29] Unfortunately, biocatalysis applications for these enzymes have been limited in part because of the oxygen sensitivity of these enzymes and also because nearly all iron(II) α-ketoglutarate halogenases identified to date require substrates to be tethered to carrier proteins of polyketide synthase or nonribosomal synthase assembly line enzymes.[3a] However, an iron(II) α-ketoglutarate halogenase that acts on a freestanding small-molecule substrate has recently been discovered, and may lead to the wider application of these enzymes to make new compounds.[30] Moreover, it has been demonstrated that the iron(II) α-ketoglutarate halogenases can perform additional functionalization reactions. In the presence of NO_2^- or N_3^- anions wild-type SyrB2, an iron(II) α-ketoglutarate halogenase from *Pseudomonas syringae* B301D, catalyzes nitration and azidation of an aliphatic carbon, albeit with modest yields.[29] A mutation of A118G in SyrB2 enlarges the active site improving the C-N coupling efficiency by 30-fold compared to wild-type enzyme.[29] Given how difficult it is to derivatize aliphatic carbons, it is well worth investing research into the biocatalytic capabilities of this class of enzymes.

Substrate recognition and regioselectivity continue to hamper the biotechnological potential of these enzymes. Identification or evolution of halogenases that act at the late stages of a metabolic pathway would mitigate the problems associated with recognition of unnatural biosynthetic intermediates by downstream biosynthetic enzymes. Most engineering efforts have focused on the regioselective flavin-containing halogenases. However, the identification of site-selective vanadium dependent chloroperoxidases may renew metabolic engineering efforts using haloperoxidase biocatalysts.[9, 31] Finally, development of novel high-throughput screening methods will also aid engineering efforts to evolve halogenases into biocatalysts.[32]

Presently chemical synthesis remains the predominate method of generating large quantities of halogenated material.[1] However, synthetic biology and metabolic engineering developments have provided an opportunity to produce a wide variety of halogenated molecules using biocatalysis. As halogenases continue to be discovered and engineered, we envision that biocatalysis will become a widely used approach for synthesizing haloorganic compounds.

Acknowledgements

Entry for the Table of Contents

CONCEPT

Halogenation has a profound impact on the biological activity of molecules. However, synthetic approaches that introduce the halogen functional group into complex molecules can be challenging to implement. Thus, the enzymes that catalyze halogenation have been subjected to intense engineering efforts to allow these enzymes to be used generally on a large scale. Here we explore the potential and limitations of halogenases as biocatalysts.

R ⟹ R—X

halgenase

X = F, Cl, Br, I
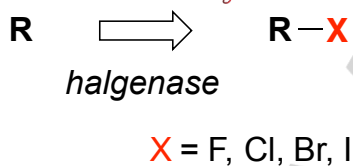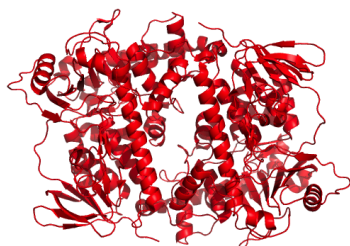
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