Catalytic mechanisms, basic roles, and biotechnological and environmental significance of halogenating enzymes

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The understanding of enzymatic incorporation of halogen atoms into organic molecules has increased during the last few years. Two novel types of halogenating enzymes, flavin-dependent halogenases and α-ketoglutarate-dependent halogenases, are now known to play a significant role in enzyme-catalyzed halogenation. The recent advances on the halogenating enzymes RebH, SyrB2, and CytC3 have suggested some new mechanisms for enzymatic halogenations. This review concentrates on the occurrence, catalytic mechanisms, and biotechnological applications of the halogenating enzymes that are currently known.

Keywords haloperoxidases; flavin-dependent halogenases; αKG-dependent halogenases; fluorinase; genetic algorithm

Over 4500 halogenated natural products are known to be produced by living organisms [1]. These products display distinct physiological or biochemical roles, for example, thyroxine functions as a hormone in mammals [2], 4-chloroindolyl-3-acetic acid is a plant growth hormone [3], and thienodolin also acts as a plant growth regulator [4,5]. Several halometabolites, particularly those of marine origin, appear to have a defensive role [6], and some are medically valuable and include antibiotics (chlorotetracycline and vancomycin), antitumor agents (rebeccamycin and calicheamycin), human thyroid hormone (thyroxine) [2], and anti-HIV agents (chloropeptin I, ambigol A) [7,8]. Commonly, the halogen that is incorporated into a particular organic substrate is determined by the relative amount of halide present in the surrounding environment. For natural organohalogen compounds found in the marine environment, bromine (Br) mostly dominates over chlorine (Cl), but for natural organohalogens found in the terrestrial environment, Cl dominates over Br [9]. The mechanism of enzymatic halogenation has become a hot topic to organic and medicinal chemists. This is because the mechanisms represent potential novel pathways to both new halogenated synthetic compounds and modified natural products.

Herein we will focus on the catalytic mechanisms, basic roles, and biocatalytic potential of halogenating enzymes.

Haloperoxidases

For the catalysis of halogenation reactions, haloperoxidases require hydrogen peroxide (H2O2) and halide ions (Cl−, Br−, or I−, but not F−) and are thus named chloroperoxidases (CPO). CPO might also use chlorite (ClO2−) instead of Cl− and H2O2 to form the halogenated products [9]. Haloperoxidases differ by the metal ion associated with the prosthetic group and mostly contain either heme iron or a vanadate co-factor for their halogenating activity [5,10]. Biochemical characterization showed that CPO (EC 1.11.1.10) from Caldariomyces fumago contains a heme group, is able to show catalytic activity, and additionally catalyzes P450-type reactions [11]. Elucidation of the 3-D structure [12] revealed the reaction mechanisms showing that heme-type haloperoxidases produce free hypohalous acids (HOX; X=Cl−, Br−, or I−) as the halogenating agent [13,14]. Recently, a second fungal haloperoxidase, Agrocybe aegerita peroxidase (AaP) (EC 1.11.1.16), of the heme-thiolate type has been discovered in the agaric mushroom A. aegerita. The AaP has strong brominating as well as weak chlorinating and iodating activities, and catalyzes both benzylic and aromatic hydroxylations (e.g., of toluene and naphthalene) [14]. Several other heme peroxidases (in addition to CPO)
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possess halogenating side activities, for example, lignin [15], manganese [16], soybean [17], and horseradish [18] peroxidase show brominating and iodating activities [14]. There are several human/animal heme peroxidases that can oxidize halides, for example, the flavin-heme CPO from the marine polychaete Notomastus lobatus [19], myeloperoxidase [20] and eosinophil peroxidase [21] from human leukocytes, as well as bovine lactoperoxidase [22], and human thyroid peroxidase [23].

Vanadium-containing haloperoxidases have been isolated from marine algae, lichen, and fungi [24], and also produce hypohalous acids as the halogenating agent [5,13,25]. A quantum mechanics/molecular mechanics study of the rest state of the vanadium-dependent CPO (EC 1.11.1.-) from Curvularia inaequalis and of the early intermediates of the halide oxidation was reported recently [26]. The investigation of different protonation states indicates that the enzyme likely consists of an anionic $\text{H}_2\text{VO}_4^-$ vanadate moiety where one hydroxyl group is in the axial position. The hydrogen peroxide directly attacks the axial hydroxyl group, resulting in the formation of a hydrogen peroxide intermediate. This intermediate is promptly protonated to yield a peroxo species (Fig. 2) [26]. The most likely protonation states of the peroxo co-factor are neutral forms $\text{HVO}_2\text{O}_2$ with a hydroxyl group either H-bonded to Ser$^{\text{402}}$ or coordinated to Arg$^{\text{360}}$. The calculations strongly suggest that the hydrogen peroxide binding might not involve an initial protonation of the vanadate co-factor, and the halide oxidation might take place with the preliminary formation of a peroxovanadate/halogen adduct (Fig. 2). Subsequently, the halogen reacts with the peroxo moiety, yielding a hypohalogen vanadate [26]. The use of haloperoxidases as halogenating biocatalysts is limited because they have in common a lack of both substrate specificity and regioselectivity.

Flavin-dependent Halogenases

Although a number of flavin-dependent halogenases have been investigated in some detail, halogenating activity in vitro has only been shown for the flavin adenine dinucleotide (FAD)-dependent tryptophan 7-halogenase (PrnA) (EC 1.14.13.2) [27,28] and PrnC [27] from pyrrolnitrin biosynthesis in Pseudomonas fluorescens Bi915, RebH from rebeccamycin biosynthesis in Lechevalieria aerocolonigenes [29], PyrH from pyrroindomycin biosynthesis in Streptomyces rugosporus [30], Thal from the thienodolin producer S. albogriseolus [31], PltA from pyoluteorin biosynthesis in P. fluorescens Pf-5 [32], and HalB from the pentachloropseudilin producer Actinoplanes

Fig. 1 Reaction mechanism of heme-haloperoxidases in halogenation [14] A-H, organic substrate; X=Cl, Br, or I.

Fig. 2 Basic reaction mechanism of vanadium-haloperoxidases in halogenation [5,13,26] A-H, organic substrate.
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sp. ATCC 33002 [33]. All of the flavin-dependent halogenases require reduced FADH$_2$ (provided by a partner flavin reductase), chloride ion, and oxygen as co-substrates for halogenation reaction [34]. The reaction of FADH$_2$ and O$_2$ in the halogenase active site was presumed to form a typical 4α-hydroperoxyflavin (FAD-4α-OOH) intermediate [34,35]. Two reaction mechanisms have been proposed for the flavin-dependent halogenases. One is the nucleophilic mechanism, suggesting the initial formation of an epoxide [27] or the addition of a hydroxyl group [36] from the substrate’s reaction with the FAD-4α-OOH intermediate. This would then be followed by the nucleophilic attack of a halide ion (chloride or bromide), leading to the formation of a halohydrin [34]. The other is the electrophilic mechanism that proposed the reaction of the FAD-4α-OOH intermediate with chloride ion to form FAD-4α-OCl [5,35]. Attack of the aromatic π electrons on the FAD-4α-OCl intermediate would lead to formation of a chlorinated substrate intermediate that, after deprotonation, would give the chlorinated product [34,35].

The elucidation of the 3-D structure [28] of PrnA involved in pyrrolnitrin biosynthesis suggests that neither the nucleophilic nor the electrophilic mechanism is correct [34]. The crystal structure of PrnA has been resolved and it indicates that the protein is composed of two modules, an FAD binding module and a tryptophan binding module [5,28]. The structure reveals that the initially formed FAD-4α-OOH cannot interact directly with the substrate tryptophan because the bound tryptophan lays 10 Å from the FAD. On the basis of this finding, the catalytic mechanism of PrnA was proposed, illustrated in Fig. 3. After formation of a FAD-4α-OOH intermediate, hypochlorous acid (HOCl) will be produced by nucleophilic attack of Cl$^-$ on FAD-4α-OOH (Fig. 3). K79 provides a hydrogen bond to the HOCl, positioning it in the correct orientation to react with the tryptophan 7-position. Furthermore, the Wheland intermediate formed during the electrophilic addition of chlorine to tryptophan is stabilized by a glutamate residue in the substrate binding site (E346), that deprotonates the intermediate yielding 7-chlorotryptophan (Fig. 3). Site-directed mutagenesis experiments showed the importance of these residues to the activity of PrnA: an E346→Q346 mutation significantly affects turnover, and a K79→A79 mutation destroys activity completely [28].

RebH is another tryptophan 7-halogenase that catalyzes the formation of 7-chlorotryptophan as the initial step in the biosynthesis of antitumor agent rebeccamycin [29,35]. Both structural and kinetic evidence of PrnA and RebH support the subsequent formation of HOCl in the active site when FAD-4α-OOH is captured by Cl$^-$ [28,37]. However, two observations from the RebH reaction kinetics and the RebH structure seemed to challenge the suggested mechanism of PrnA. First, during stopped flow studies to monitor formation of flavin intermediates in RebH, flavin reactions leading to HOCl production were observed with or without L-Trp present, suggesting that this potent oxidant is formed in the active site without available substrate for reaction [35]. Second, in the crystal structure of RebH with bound flavin and tryptophan solved at 2.1 Å, Lys$^{79}$ occupies a key position between the binding pockets for flavin and substrate tryptophan (corresponding to the same residue in PrnA) [28,37]. Studies of protein oxidation by HOCl show that the εNH$_2$ of lysine reacts

Fig. 3 Mechanism of tryptophan chlorination catalysed by tryptophan 7-halogenase [28]  
Formation of HOCl by attack of Cl$^-$ on flavin peroxide and electrophilic chlorination of tryptophan.
rapidly with HOCl to form a long-lived chloramine, Lys-εNH-Cl ($t_{1/2} > 25$ h) [37,38]. Chloramines can also carry out chlorination reactions [39−41], and might play an important role in the flavin halogenase mechanism [42,43]. Lys-εNH-Cl was formed in the RebH active site when the reaction of FADH$_2$, Cl$^-$, and O$_2$ was catalyzed in the absence of substrate tryptophan, and the chlorinating species is remarkably long-lived with $t_{1/2}$ of 63 h at 4 ºC and 28 h at 25 ºC [37]. Based on these observations, a challenge to the mechanism of PrnA was put forward by Yeh et al [37] in a different mechanism that HOCl reacts with the active site Lys$^{79}$ of RebH to form a lysine chloramine Lys-εNH-Cl before reaching the substrate tryptophan (Fig. 4). This intermediate remained on the enzyme after removal of FAD and transferred chlorine to tryptophan with kinetically competent rates (Fig. 4). Furthermore, a similar chlorinating species has also been detected in the halogenase PltA from pyoluteorin producer P. fluorescens Pf-5 [37]. Three proteins (PltA, PltD, and PltM) involved in pyoluteorin biosynthesis [34] are homologous to FADH$_2$-dependent halogenases found in other non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) biosynthetic gene clusters [32]. Assay of halogenating activity with L-pyrrolyl-S-PltL as the substrate in vitro revealed that only PltA catalysed the incorporation of both chlorine atoms [44].

All flavin-dependent halogenases have two conserved motifs (Fig. 5). The first motif (GxGxxG), which is the FAD-binding site, is located near the N-terminus [30], and is also known to be involved in the binding of nucleotide co-factors of the large family of protein kinases [45]. However, in PltD this motif is not absolutely conserved (GxSxxV), it is only a halogenase-like protein of unknown function [34,46]. The second absolutely conserved motif located near the middle of the enzymes contains two tryptophan residues (WxWxIP) (Fig. 5). Again, this motif is not absolutely conserved in PltD (WxGxIP), showing that this enzyme is not a halogenase [34]. The two tryptophan residues of this motif are located near the flavin, and they are suggested to block the binding of a substrate close to the flavin and thus prevent the enzyme from catalysing a monoxygenase reaction [28,30].

### α-Ketoglutarate (αKG)-dependent Halogenases

A class of αKG-dependent halogenases responsible for halogenation of unactivated carbon centers in the biosyntheses of several compounds of non-ribosomal peptide origin has recently been characterized [47−53]. Unlike haloperoxidases and flavin-dependent halogenases, this novel type of halogenase does not require a substrate with a double bond for introduction of halogen atoms [34]. Studies showed that the in vitro reconstitution of the aliphatic halogenation activity of these enzymes requires halogenase, Fe$^{II}$, and three small-molecule co-substrates, αKG, oxygen, and chloride [48,49]. Halogen incorporation follows the consensus mechanism of non-ribosomal peptide biosyntheses, an amino acid will be used as its initial substrate and initial activation of the amino acid by

![Fig. 4 Mechanism of tryptophan chlorination catalysed by halogenating enzyme RebH from rebeccamycin biosynthesis in Lechevalieria aerocolonigenes [37]](image)  
HOCI reacts with the active site Lys$^{79}$ of RebH to form a lysine chloramine Lys-εNH-Cl before reaching the substrate tryptophan.
an adenylation (A) domain is followed by its loading on the phosphopantetheinyl arm of the thiolation (T) module. The resultant aminoacyl-S-T protein is the substrate for the halogenase, which chlorinates an unactivated methyl group of the tethered amino acid [50]. For example, chlorination of the methyl group of L-threonine tethered to the A-T didomain protein SyrB1 by the halogenase SyrB2 produces 4-chloro-L-threonine-S-SyrB1, an intermediate in the biosynthesis of the antifungal agent syringomycin E [Fig. 6(A)] [49]. Similar chlorination also occurs in the biosynthesis of the non-halogenated phytotoxin coronatine in P. syringae pv. tomato [51].

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\alpha \text{KG-dependent halogenase CmaB chlorinates the L-allo-isoleucine to form the } \gamma\text{-Cl-L-allo-isoleucine, an intermediate in the formation of the cyclopropane ring of CMA, a substrate for coronatine biosynthesis [52,53]. CytC3, the halogenase isolated from soil Streptomyces sp., chlorinates the methyl group of L-2-aminobutyric acid (L-Aba) or L-valine tethered to the carrier protein CytC2 in [Fig. 6(C)] [54]. BarB1/BarB2 and DysB1/DysB2 have been suggested to be the halogenases catalysing the chlorinating reactions in barbamide and disidenin/dysideathiazole biosynthesis, respectively [47,50].}

However, the mechanism of such aliphatic halogenations has not been elucidated. Insight into the catalytic strategy of Fe\(^{II}/\alpha\text{KG}\)-dependent halogenases came from the crystal structure of the syringomycin halogenase SyrB2 [48]. In contrast to the \(\alpha\text{KG}\)-dependent dioxygenases, the Fe center of SyrB2 is coordinated by two protein-derived histidines, bidentate \(\alpha\text{KG}, \text{water, and chloride. The carboxylate of the “facial triad” that normally coordinates the Fe\(^{II}\) center is replaced with an alanine in the protein primary structure, presenting a coordination site for the chloride ligand [54]. On the basis of this observation, the mechanism of the Fe\(^{II}/\alpha\text{KG}\)-dependent halogenases shown in Fig. 7 was proposed [47,48,50]. The early steps of the mechanism leading to the ClFe\(^{IV}\)-oxo complex are likely conserved among the dioxygenases and halogenases [50]. The key postulated intermediate ClFe\(^{IV}\)-oxo complex activates the substrate by hydrogen atom abstraction to yield a ClFe\(^{III}\)-OH complex and a substrate radical (Fig. 7). Substrate chlorination was proposed to proceed through “rebound” of a chloride radical, rather than the hydroxyl radical rebound postulated for hydroxylases [50,54]. Exclusive halogenation (rather than hydroxylation) reflects the lower reduction potential of chlorine radical (Cl •+e−→Cl−, 1.36 V) relative to hydroxyl radical (HO•+e−→HO−, 2.02 V) [54]. The proposed mechanism of Fe\(^{II}/\alpha\text{KG}\)-dependent halogenases was tested experimentally by direct characterization of the intermediates (ClFe\(^{IV}\)-oxo complex) using a combination of kinetic and spectroscopic methods [54] in the aliphatic halogenase CytC3 from soil Streptomyces sp. [50].

**Fluorinase**

5′-Fluoro-5′-deoxyadenosine (5′-FDA) synthase (EC 2.5.1.63) isolated from Streptomyces cattleya is the first fluorinating enzyme [5,55]. The fluorinase gene (flA) has been characterized recently, and 11 other putative open reading frames have been identified [56]. Three of the proteins encoded by these genes have also been characterized. FIB was the second enzyme in the biosynthetic pathway of fluorometabolites, catalyzing the phosphorolytic cleavage of 5′-FDA to produce the next intermediate 5-fluoro-5-deoxy-D-ribose-1-phosphate [57]. Fluoroacetaldehyde combines with the amino acid L-threonine in a pyridoxal phosphate-dependent transaldol reaction to generate the antibiotic 4-fluorothreonine. In a separate reaction fluoroacetaldehyde is oxidised to fluoro-
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Fig. 6 Initial steps in the biosynthetic pathways of (A) syringomycin E [49], (B) coronatine [51], and (C) cytotrienin [54] All begin with attachment of amino acid to an adenylation/thioesterase domain (SyrB1, CmaA and CytC2), followed by chlorination (SyrB2, CmaB, and CytC3). A, adenylation domain; T, thiolation module.

Fig. 7 Proposed mechanism of chlorination by the FeII/αKG-dependent enzymes [42] A, adenylation domain; T, thiolation module.
acetate by the action of an NADH-dependent aldehyde dehydrogenase. A summary of the fluorometabolite pathway is shown in Fig. 8 [58]. The enzyme FlI is an S-adenosylhomocysteine hydrolase that might act to relieve S-adenosylhomocysteine inhibition of the fluorinase. Finally, FlK was proposed for the specific degradation of fluoroacetyl-co-enzyme A into fluoroacetate and co-enzyme A (Fig. 8) [56]. The fluorinase from S. castellana is also a chlorinase, and can also use Cl\(^{-}\) as a substrate generating 5\'-chloro-5\'-deoxyinosine (Fig. 8) [59]. The reactions with both fluoride and chloride are reversible (Fig. 8) [55,58,59]. A mechanism study that used stereospecifically-labeled S-adenosyl methionine carrying deuterium at the 5\'-pro-S site revealed that 5\'-FDA synthase catalyses the synthesis of 5\'-FDA from S-adenosyl methionine and fluoride by an 5\',5\'' substitution reaction that also occurs in the biosynthesis of 5\' Chloro-5\'-deoxyinosine [60].

**Optimisation of Halogenase Enzyme Activity by Genetic Algorithm**

5-Hydroxytryptophan (5-HTP) is a component of many antidepressant drugs. Commonly it is obtained by seed extraction of the African plant Griffonia simplicifolia. The bioanalytical system shown in Fig. 9 could also generate 5-HTP for pharmaceutical and fine chemical applications [61]. However, the production rate of the enzyme-producing bacteria and the activity of the purified enzyme are too low for efficient application in the production of 5-HTP. To overcome the supply problem, a genetic algorithm (GA) was applied for a tryptophan-5-halogenase activity assay formulation for enzyme activity optimization that, in this special case, is influenced by six different factors/parameters [62]. The GA makes an optimization step within a cycle of four stages: creation of a population of individuals (experiments); evaluation of these experiments; selection of best experiments and breeding; and, aided by genetic manipulation, creation of a new population. Real variables are generally encoded in the form of binary character strings. For a better performance, all parameters were encoded according to the Gray code application for the binary bit

![Fig. 8 Biosynthetic pathway to the fluorometabolites fluoroacetate and 4-fluorothreonine [56–59]](image1)

![Fig. 9 Hypothetical reaction sequence for manufacturing 5-hydroxytryptophan [62]](image2)

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These investigations have shown that such an approach rebeccamycin genes with selected tryptophan halogenase compounds has been produced by co-expression of product. A series of novel chloro-indolocarbazole genesis could lead to a diverse range of halogenated organic substrates and a sensible program of mutational substitution incorporating Co, Ni, Zn, and Cu [9]. Synthetic haloperoxidases have been prepared by metal compounds to be used in industrial catalytic conversions. The vanadium-dependent chloroperoxidase might help in molecular mechanics study of the rest state of the [14]. The new findings from the quantum mechanics/molecular mechanics study of the vanadium-dependent chloroperoxidase might help in understanding the action mechanism of enzymes and give precious new insights for the design of biomimetic compounds to be used in industrial catalytic conversions. Synthetic haloperoxidases have been prepared by metal substitution incorporating Co, Ni, Zn, and Cu [9].

The genes of flavin-dependent halogenases have been identified in the biosynthetic gene clusters of structurally very different compounds. In theory, halogenating a range of organic substrates and a sensible program of mutagenesis could lead to a diverse range of halogenated product. A series of novel chloro-indolocarbazole compounds has been produced by co-expression of rebeccamycin genes with selected tryptophan halogenase genes rebH, pyrH, and thal from other microorganisms in the Streptomyces albus expression system [Fig. 10(A,B)] [5,64]. Transformation of the pyrrolnitrin producer P. chlororaphis ACN with a plasmid containing the thal gene led to the formation of the new aminopyrrolnitrin derivative 3-(2’-amino-4’-chlorophenyl) pyrrole [Fig. 10(C)] [5,31]. These investigations have shown that such an approach to generating halogenated analogs of biologically active compounds is feasible, and as more halogenases are discovered, the range of applications will increase. Detection of the long-lived chlorinating intermediate in the flavin-dependent halogenase mechanism suggests nature’s ingenious solution to the chemical problem of controlling a reactive and potentially destructive oxidant, HOCl, for C-Cl bond construction [37]. αKG-dependent halogenases have no problem with reactive power, but the system is complicated by the requirement of the adenylation/thioesterase component for turnover [48–51,54]. If αKG-dependent halogenases could be engineered to accept the untethered substrate, a whole range of chemistry would be opened up.

In contrast to haloperoxidases, halogenases (e.g. flavin-dependent halogenases, αKG-dependent halogenases) are capable of catalysing the regioselective formation of carbon halogen bonds and are therefore of particular interest for applications in white biotechnology, as toxic halogenating agents could be substituted through the less harmful halides [27], and fewer by-products are produced [65]. Due to their application as intermediates in palladium (Pd)-catalysed carbon coupling reactions they are also of tremendous interest for the production of organic fine chemicals [62]. With the fluorinase gene (flA) characterized, there are clear opportunities to clone it into other micro-organisms to “kick start” organo-fluorine metabolite production in other organisms [55]. Huang et al. have identified a cluster of approximately 10 genes that most likely express proteins involved in the biosynthesis of the fluorometabolites of S. cattleya [56]. It is attractive to consider inserting all of these genes as a cassette into candidate alternative organisms to assess if that initiates the biosynthesis of novel organo-fluorine compounds from such engineered microorganisms. The application of stochastic search strategies (e.g. GAs) is well suited to fast determination of the global optimum in multidimensional search spaces, where statistical approaches or even the popular classical one-factor-at-a-time method often fails by misleading to local optima. Biotransformations to halogenated starting materials and building blocks from inorganic halogen represent novel territory in organohalogen chemistry and merit investigation.

A large number of halogenated compounds are produced by chemical synthesis. Some of these compounds are very toxic and cause enormous problems to human health and to the environment. Investigations on the degradation of halocompounds by microorganisms have led to the detection of various dehalogenating enzymes catalyzing the removal of halogen atoms under aerobic and anaerobic
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Fig. 10 Combinatorial biosynthesis of indolocarbazole compounds [64] and new intermediate 3-(2′-amino-4′-chlorophenyl)pyrrole (6-Cl-MCAP) [31]

(A) Pathways constructed by dissection and reconstitution of rebeccamycin biosynthesis or coexpression of rebeccamycin and staurosporine genes. (B) Pathways constructed by coexpression of rebeccamycin, staurosporine, pyrroindomycin, and thienodolin genes. (C) Formation of the new intermediate 6-Cl-MCAP of pyrrolnitrin biosynthesis by combinatorial biosynthesis. PyrH, tryptophan 5-halogenase from Streptomyces rugosporus; RebC, FAD-containing monoxygenase; RebD, chromopyrrolic acid synthase; RebF, flavin reductase; RebG, N-glycosyltransferase; 7-Chloro-Try, tryptophan 7-halogenase; RebM, sugar O-methyltransferase; RebO, amino acid oxidase; RebP, P450 oxygenase from L. aerocolonigenes; StaC, FAD-containing monoxygenase from S. longisporoflavus; Thal, tryptophan 6-halogenase from S. albogriseolus.

conditions involving different mechanisms [13]. The NADH-dependent enzyme maleylacetate reductase, which catalyzes a different type of reductive dehalogenation reaction, has been isolated from several Pseudomonas strains and Ralstonia eutropha [66]. The question whether there is any connection between biological halogenation and dehalogenation, still can not be answered. There are no data available showing that biological halogenation led to the development of dehalogenating enzymes [13]. Additionally, similarities between halogenating and dehalogenating enzymes have not yet been found. The search for a halogenating enzyme also led to the development of dehalogenating enzymes (e.g. CmaC from P. syringae) [53].
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References

5 Xian-Ping C. Formation of novel tryptophan-derived compounds by combinatorial biosynthesis using regioselective tryptophan halogenases (Master’s thesis). Dresden: Dresden University of Technology 2006
13 van Pée KH, Unversucht S. Biological dehalogenation and halogenation reactions. Chemosphere 2003, 52: 299–312
17 Munir IZ, Dordick JS. Soybean peroxidase as an effective bromination catalyst. Enzyme Microb Technol 2000, 26: 337–341
23 Rawitch AB, Taurog A, Chernoff SB, Dorris ML. Hog thyroid peroxidase: physical, chemical, and catalytic properties of the highly purified enzyme. Arch Biochem Biophys 1979, 194: 244–257
34 van PEE KH, PATALLO EP. Flavin-dependent halogenases involved in secondary metabolism in bacteria. Appl Microbiol Biotechnol 2006,


