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Scope and potential of halogenases in biosynthetic applications

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A large and diverse series of halogenated natural products exist. In many of these compounds the halogen is important to biological activity and bioavailability. We now recognise that nature has developed many different halogenation strategies for which well-known enzyme classes such as haem oxidases or flavin-dependent oxidases have been adapted. Enzymes capable of halogenating all kinds of different chemical groups from electron-rich to electron-poor, from aromatic to aliphatic have been characterised. Given that synthetic halogenation reactions are not trivial transformations and that halogenated molecules possess pharmaceutical usefulness, it will be worth investing into further research of halogenating enzymes.

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Introduction

A large and diverse series of halogenated natural products exist, with the number identified by 2010 exceeding 4700 [1]. These compounds, initially considered nothing more than an oddity, attract interest because of their structures, their biogenesis and their biological activity [2]. In many of these compounds the halogen is important to biological activity and bioavailability. Halogens are also found in biosynthetic intermediates where they act as facilitators for chemical reactions such as the cyclopropane ring formation in the marine natural product curacin (Figure 1) [3]. The most common halogen found in natural products is chlorine followed by bromine, whilst iodine and fluorine are considerably rare [4]. The occurrence of brominated compounds is more frequently observed in marine natural products. In terms of drug discovery and development, chlorinated and brominated compounds have the merit of being amenable to specific chemical modification using the halogen as a functionalisable chemical handle [5] in addition to modulating biological activity and physical properties. Fluorination is a particularly sought-after halogenation with an excellent track record of increasing drug efficacy through numerous mechanisms [6].

For many years the only known halogenases were the haloperoxidases. We now have a much better understanding of enzymatic halogenation. In the past decade, a veritable explosion of new halogenation mechanisms and enzymes has come to light. Among them are flavin-dependent halogenases and α-ketoglutarate-dependent non-haem iron oxygenases that have modified well known reaction mechanism to perform chlorination and bromination reactions; and *S*-adenosylmethionine-utilising (AdoMet-utilising) enzymes that are capable of fluorination or chlorination through a nucleophilic mechanism [7,8].

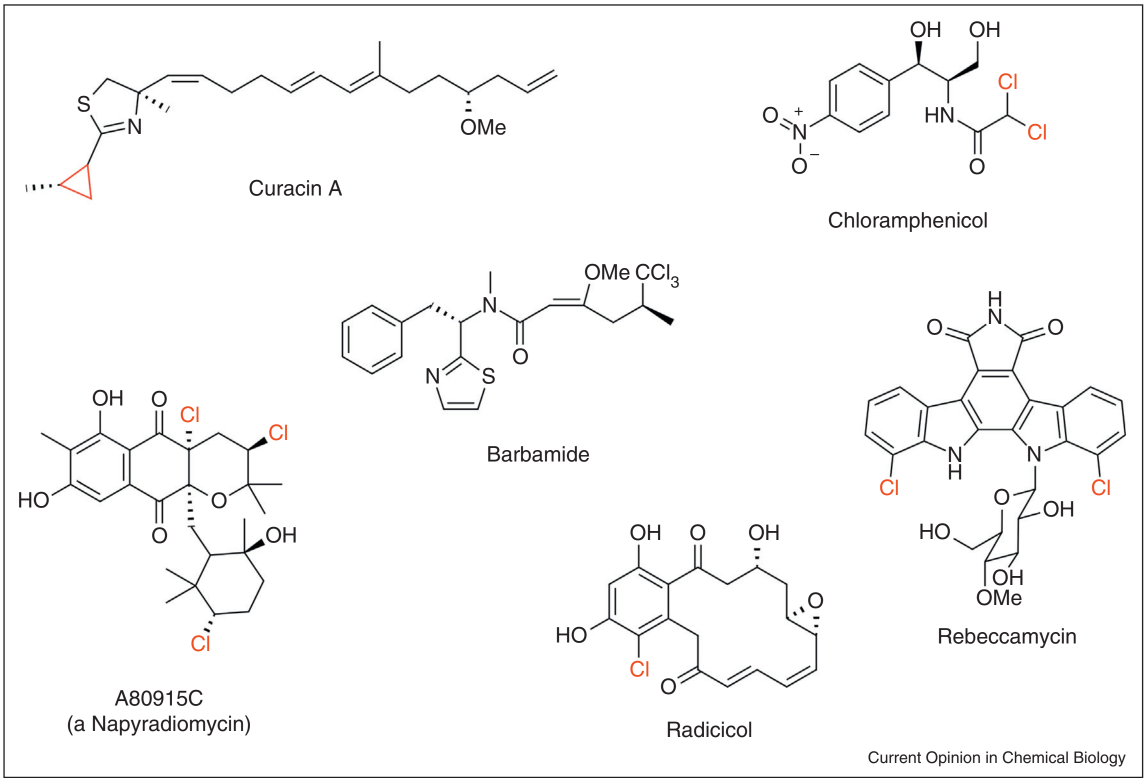
Haloperoxidases

Haloperoxidases have been known for a very long time as halogenating enzymes. One example is thyroid peroxidase which is involved in thyroxine biosynthesis [9]. The two major classes comprise haem iron peroxidases and vanadium peroxidases. The electrophilic halogenating species generated by these enzymes is likely to be hypohalous acid, a highly reactive halogenating agent. Chlorination, bromination as well as iodination are observed with this family of halogenases; however, no fluorination activity has yet been demonstrated, mostly likely due to the high electronegativity of fluorine.

The general mechanism of haloperoxidases involves the generation of the halogenating species, through oxidation of the halide, and is dependent on hydrogen peroxide (Figure 2a). The reaction mechanism of haem peroxidases is likely to parallel that of other haem enzymes [10,11]. The halide ion is oxidised in the active site to ferric hypohalite by the ferryl-oxo species. This species in turn is generated through binding of hydrogen peroxide to the ferric resting state (Figure 2c). Similarly, in vanadium-dependent halogenases hydrogen peroxide binds to the metal which is followed by halide addition and finally the release of the hypohalous acid (Figure 2b) [8,12].

Haloperoxidases often exhibit low regiospecificity due to the freely diffusing halogenating agent that is generated and due to the high reactivity of hypohalous acid; as a result a suite of various monohalogenated, dihalogenated and trihalogenated metabolites can be generated. Studies on the bromination of methyl pyrrole-2-carboxylate using a vanadium-dependent haloperoxidase from the alga

Figure 1



Examples of halogenated natural products.

Ascophyllum nodosum gave mixtures of 4-bromopyrroles and 5-bromopyrroles as well as 4,5-dibromocompounds [13]. However, successful applications with haloperoxidases have been demonstrated. For example, the bioprocess department of Merck demonstrated that indene oxide, an intermediate in the synthesis of the HIV-1 protease inhibitor Crixivan® (Indinavir) can be obtained through biotransformation [14].

It can be argued that many of the chloroperoxidases or bromoperoxidases described in the literature are not halogenases by primary function but the observed halogenating activity stems from the general ability of peroxidases to oxidise halides when taken out of biological context. Non-native halogenase activity could be the cause of HOCl being released from the enzyme active site. This could also explain the apparent lack of stereoselectivity and regioselectivity of some haloperoxidases that seem to contradict the specific halogenation patterns in natural products.

The existence of highly regiospecific and stereospecific vanadium-dependent haloperoxidases such as Mcl24 of the merochlorin pathway (speculative) and NapHI of the napyradiomycin pathway implies that the highly reactive nature of the postulated hypohalous acid reagent can be moderated and that these enzymes do indeed bind their substrates in a highly specific fashion [15,16•,17].

Flavin-dependent halogenases

The discovery of PrnA, a flavin-dependent halogenase from the pyrrolnitrin biosynthesis, demonstrated that alternative, more specific mechanisms of halogenation existed [18]. The exact mechanism with which flavin-dependent halogenases operate is still under debate, and mechanistic details, such as which active site residues participate, may actually vary between members of the family. The reader is referred to a number of excellent reviews on the topic [8,19–21]. Halogenation with flavin-dependent enzymes involves an electrophilic species such as hypohalous acid which is generated from the reaction of reduced flavin with molecular oxygen (Figure 2d). The reduced flavin in turn is provided by an additional enzyme, flavin reductase. Most halogenases bind the flavin co-factor non-covalently; however, studies on CmlS from the chloramphenicol biosynthesis have demonstrated that the flavin co-factor can also be covalently attached to the enzyme [21]. The most notable feature in flavin-dependent halogenases is that the flavin binding site and the substrate binding site are separated by a 10 Å long tunnel through which the hypohalous acid