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Unusual flavoenzyme catalysis in marine bacteria

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Ever since the discovery of the flavin cofactor more than 80 years ago, flavin-dependent enzymes have emerged as ubiquitous and versatile redox catalysts in primary metabolism. Yet, the recent advances in the discovery and characterization of secondary metabolic pathways exposed new roles for flavin-mediated catalysis in the generation of structurally complex natural products. Here, we review a selection of key biosynthetic flavoenzymes from marine bacterial secondary metabolism and illustrate how their functional and mechanistic investigation expanded our view of the cofactor's chemical repertoire and led to the discovery of a previously unknown flavin redox state.

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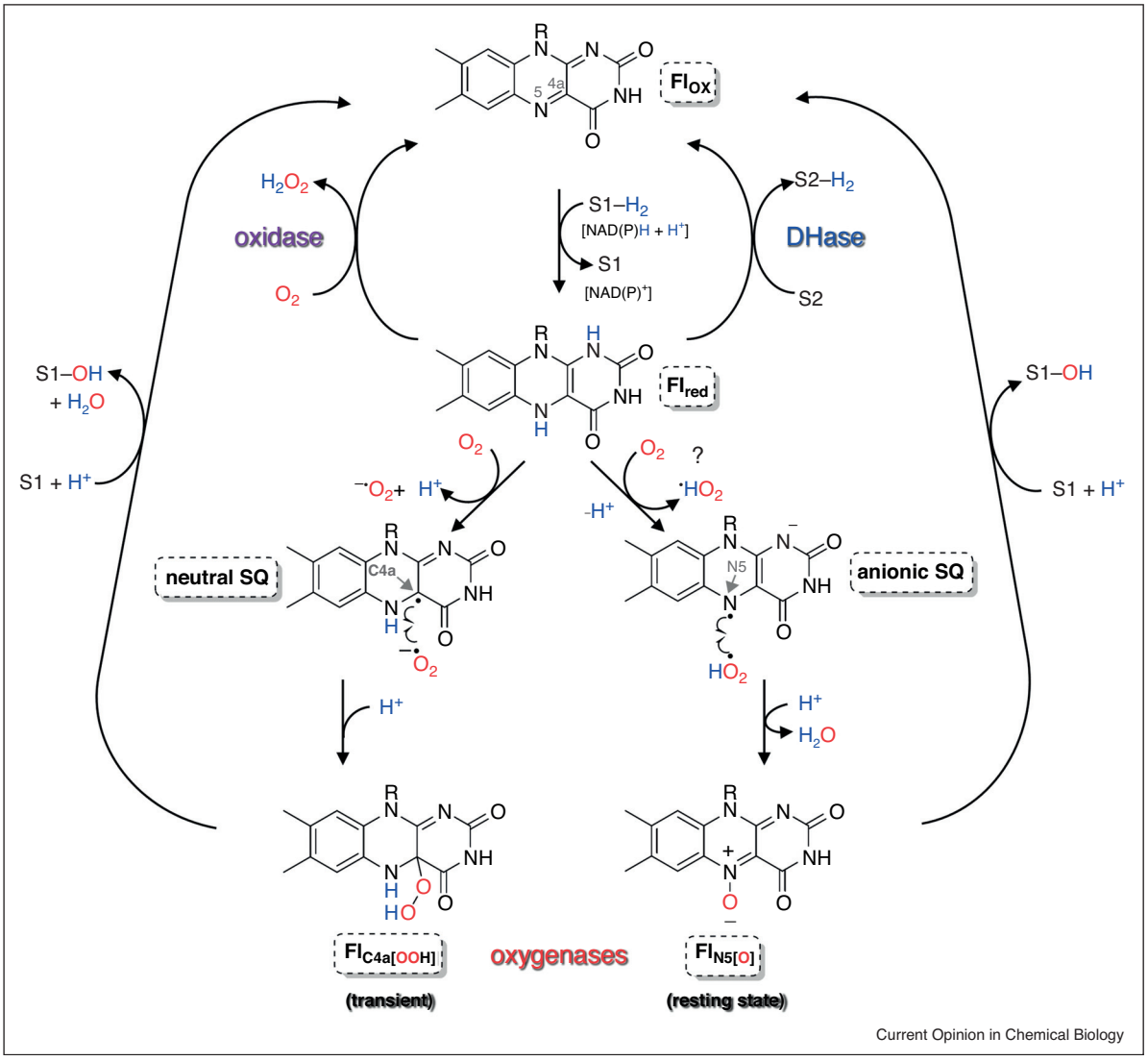
Introduction

Various marine organisms, ranging from invertebrates and plants to microbes, produce structurally diverse secondary metabolites with a vast potential for medical application (e.g., as antibiotics or in cancer treatment) [1–3]. Marine bacteria, in particular, were recognized as prolific and underestimated producers of natural product chemicals [1,4], whose biosyntheses frequently require redox tailoring enzymes containing riboflavin (vitamin B2)-derived cofactors in the form of flavin mononucleotide (FMN) and, more frequently, flavin adenine dinucleotide (FAD) [5]. Aside from secondary metabolism, ubiquitous flavoenzymes catalyze numerous essential redox reactions in all domains of life, for example, in central metabolism, drug metabolism, immune defense, cell signaling, protein folding, DNA repair, apoptosis, light emission, and neural development [6]. The key to the unique reactivity and versatile redox chemistry of the flavin cofactor is the reactive N5-C4a locus of the isoalloxazine ring system, which serves as entry/exit points for electrons as well as a site for covalent adduct formation (Figure 1) [5,7]. Under aerobic conditions, flavins are commonly oxidized (Fl_{ox}), while radical, single-electron reduced neutral (blue, $FlH^{\bullet}$) or anionic (red, $Fl^{-\bullet}$) semiquinones (SQs) and two-electron-reduced hydroquinones (Fl_{red}) represent catalytically important redox states (Figure 1) [8•,9••,10–14]. Typical flavin-dependent reactions include the electron exchange between obligatory one-electron (e.g., Fe^{III}/Fe^{II}) and two-electron (e.g., NAD(P)H) donors/acceptors or the dehydrogenation of diverse organic substrates (Figure 1). As a rare exception among organic cofactors, protein-bound Fl_{red} furthermore enables the efficient reduction of molecular oxygen. H_2O_2-forming flavin-dependent oxidases thereby exploit O_2 as electron acceptor, as exemplified by NADPH oxidase or monoamine oxidase [13]. Flavin-dependent monooxygenases, by contrast, employ O_2-derived covalent flavin oxygen adducts as oxygenating species in the form of the flavin-C4a-peroxide ($Fl_{C4a[OOH]}$) [14] or the flavin-N5-oxide ($Fl_{N5[O]}$) [8•,9••] (Figure 1). The electrophilic character of the well-studied transiently produced $Fl_{C4a[OOH]}$ is comparable to other organic hydroperoxides [15] and allows the monooxygenation of organic substrates, for example in the *p*-hydroxybenzoate hydroxylase that catalyzes an electrophilic aromatic substitution reaction [10,16–19]. Enzyme-assisted deprotonation of the hydroperoxide, however, gives rise to a C4a-peroxyanion that serves as a nucleophilic oxygenating agent, for example, in Bayer-Villiger type oxidations of ketones to esters [20]. As a further variation of this chemistry, flavin-dependent halogenases exploit the $Fl_{C4a[OOH]}$ as oxidant for the formation of halonium ions, which add to organic substrates through electrophilic substitutions [21,22]. The recently discovered $Fl_{N5[O]}$ oxygenating species was shown to mediate an oxidative Favorskii-type carbon–carbon rearrangement and may conceivably constitute a versatile redox catalyst, albeit its chemistry is less understood at present (see discussion below) [8•,9••] (Figure 1) [5]. In this review, we highlight a small selection of recently described marine bacterial flavin-dependent redox tailoring enzymes that adopt crucial roles in the formation of structurally distinct natural products and illustrate the broad diversity of flavin-mediated catalysis. For a comprehensive overview of

Figure 1



Overview of flavin redox states and catalysis (R = ribityl-ADP (FAD) or phosphoribityl (FMN)). Oxidases and dehydrogenases (DHase) oxidize organic substrates (S1) and utilize O_2 or alternative substrates (S2) for the reoxidation of Fl_{red} to the catalytically active Fl_{ox}, respectively. Monooxygenases employ a flavin-C4a-(hydro)peroxide or a flavin-N5-oxide for the oxygenation of organic substrates (S1). The radical formation of these oxygenating species proceeds via reduction of Fl_{ox} to Fl_{red} by NAD(P)H or the substrate, which may enable the single electron reduction of O_2 and subsequent radical coupling of the formed superoxide and the flavin SQ at the spin density sites C4a or N5. Note that the tentative pathway for $Fl_{N5[O]}$ formation requires further investigation.

biosynthetic flavoenzymes, we would like to recommend the excellent work of Walsh and Wencewicz [5].

Bromination in the biosynthesis of toxic polybromophenols and polybromopyrroles

The marine environment is an exceptionally rich source of halogenated natural products [23], in particular of brominated natural products that are perhaps exclusively of marine origin. While the participation of flavoenzymes as halogenating catalysts in chlorinated natural product biosynthesis is well established (Figure 2a,b) [24], it is surprising to note that physiological marine brominating flavoenzymes, those that cannot catalyze the oxidative incorporation of chlorine [25], had evaded discovery until very recently. Querying the genetic and molecular bases for the biosynthesis of the cytotoxic [26] marine natural product pentabromopseudilin revealed the '*bmp*' gene locus that incorporates two intriguing flavin-dependent brominases [27••]. Of these, the enzyme Bmp2 catalyzes the tribromination of L-proline derived pyrrole ring that is acylated to an acyl carrier protein (ACP) (Figure 2c). It is intriguing to note that despite the presence of a 900-fold excess of chloride in sea-water, Bmp2 demonstrates extraordinary halide specificity, in that no chloride