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Flavin metamorphosis: cofactor transformation through prenylation

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Prenylated flavin (prFMN) is a recently discovered cofactor that underpins catalysis in the ubiquitous microbial UbiDX system. UbiX acts as a flavin prenyltransferase while UbiD is a prFMN-dependent reversible (de)carboxylase. The extensive modification of flavin by prenylation, and the consecutive oxidation to the prFMN^{iminium} azomethine ylide, leads to cofactor metamorphosis. While prFMN is no longer able to perform N5-based classical flavin chemistry, it is capable of forming cycloadducts with dipolarophiles, long-lived C4a-based radical species as well as undergoing extensive light driven isomerization. An ever-expanding range of distinct prFMN forms hints at the possibility of novel prFMN driven biochemistry yet to be discovered.

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Introduction

Flavins are arguably one of the most versatile cofactors in nature, with flavoenzymes exploiting the chemical versatility of the isoalloxazine ring system to catalyse a wide range of reactions [1,2]. In each case, the flavin-binding protein scaffold provides the appropriate chemical environment to selectively carry out the required transformation. Many of these can be categorized as redox reactions, cycling the flavin cofactor in between the oxidized, one-electron reduced and two-electron reduced forms concomitant with substrate oxidation or reduction. Certain flavoenzymes will activate oxygen to achieve oxygenase/hydroxylation chemistry [3]. However, not all flavoenzymes are oxidoreductases: some catalyse light mediated reactions [4] while others carry out covalent catalysis [5]. On occasion, the flavin cofactor itself is covalently attached to the enzyme, a modification that can serve to alter the redox potential [6]. Such modification frequently occurs on the C6 or C8 position, and does not fundamentally alter the flavin reactivity that is centered on the N5 and C4a atoms [7]. In contrast, while transient covalent modification at N5 and/or C4a features in various enzyme mechanisms, their permanent modification has traditionally been associated with covalent inhibition of flavoproteins [5]. The recently discovered formation of prenylated FMN (prFMN) by UbiX [8••], and the consequent use of prFMN as a cofactor by the UbiD (de)carboxylase [9••], illustrates that permanent N5 modification can yield a very distinct catalyst (Figure 1). In this case, prenylation at the N5 and C6 positions leads to formation of a fourth non-aromatic ring, and achieves a complete metamorphosis of the flavin cofactor into the prFMN cofactor. This review will serve to illustrate our present understanding of prFMN biosynthesis and function, and highlight areas for further study.

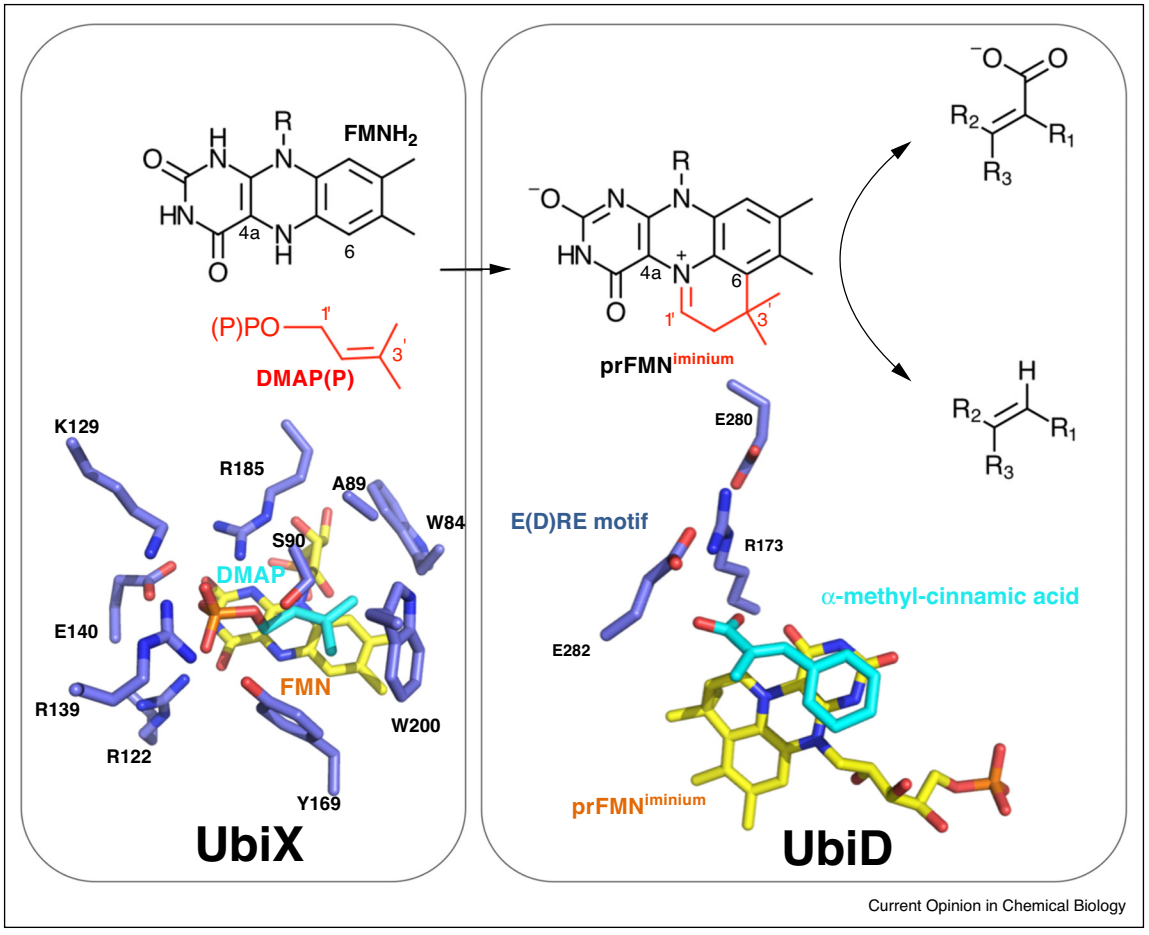
Discovery of the prFMN cofactor in the UbiDX system

The UbiDX system consists of two proteins frequently encoded within an operon/gene cluster that are associated with reversible non-oxidative decarboxylation of aromatic substrates [10,11]. Early studies hinted at the requirement of an unidentified cofactor for the canonical UbiD enzyme involved in bacterial ubiquinone biosynthesis [12], while UbiX was found to be a flavoprotein distantly related to the cysteine decarboxylase family of flavoenzymes [13,14]. The erroneous idea that both UbiX and UbiD were (redundant) decarboxylases permeated in the literature for considerable time, hence the fungal homologues of UbiX and UbiD are respectively named Pad (phenylacrylic acid decarboxylase) and Fdc (ferulic acid decarboxylase) [15]. In 2015, three papers were published that corrected this view [8••,9••,16]. The fungal Fdc/UbiD was found to be the (de)carboxylase enzyme, depending on the novel cofactor prFMN, the latter synthesized by Pad/UbiX (Figure 1). The UbiDX system is widespread in microbes, and recent biochemical studies on a range of distinct UbiD homologues — including the canonical UbiD — have established all depend on prFMN [9••,17•,18•,19•,20,21•] (Figure 2). A thorough screening of UbiX activities across the phylogenetic spectrum revealed conservation of flavin prenyltransferase functionality [22••].

Mechanism of prFMN biosynthesis

Following the identification of prFMN in fungal Fdc, the UbiX flavoprotein was postulated to act as a

Figure 1



The UbiDX system.
A schematic representation of the overall UbiDX reaction, depicting the UbiX mediated conversion of $FMNH_2$ and DMAP(P) to the prFMN cofactor. The latter is used by UbiD to catalyse (de)carboxylation of acrylic or aromatic acids. The active site structures of a representative UbiX (*P. aeruginosa* PDB code 4ZAF) and UbiD (Fdc from *A. niger* PDB code 4ZA7) are depicted adjacent to their respective reactions.

prenyltransferase [8••]. *In vitro* prFMN formation could be achieved using UbiX with $FMNH_2$ and dimethylallyl-monophosphate (DMAP) as substrates. UbiX crystal structures revealed DMAP is positioned directly above the FMN isoalloxazine ring (Figure 1), and that an intermediate N5-C1' dimethylallyl adduct is formed before formation of the C6–C3' bond (Figure 3a). Unlike many prenyltransferases [23], UbiX is not metal-dependent and the bacterial *Pseudomonas aeruginosa* UbiX was found to strictly require DMAP as opposed to dimethylallyl-pyrophosphate (DMAPP) commonly used in isoprenoid biosynthesis [24]. Common characteristics with other prenyltransferases are the formation of a so-called π-cage surrounding the substrate prenyl group. The π-cage is proposed to assist with carbocation formation, and it is interesting to note that certain flavoenzymes, such as the lycopene synthase CrtY [25], are proposed to assist with formation of a substrate carbocation by close juxtaposition with an electron rich reduced flavin.

Recent studies highlighted two possible routes for formation of DMAP in *Escherichia coli*, occurring either through hydrolysis of DMAPP via Nudix-type phosphorylases or through phosphorylation of prenol via the kinase ThiM [22••]. In fact, in certain bacterial genomes a Nudix like gene is found adjacent to UbiX [26,27]. In contrast, the yeast Pad enzyme was shown to prefer DMAPP as a substrate, suggesting some divergence in terms of prenyl group donor [28]. The close juxtaposition of DMAP(P) and reduced $FMNH_2$ is not sufficient for prenyltransfer, as demonstrated by the evolutionary and structurally unrelated flavoenzyme IDI-2 that catalyses isomerization of DMAPP into isopentyl diphosphate using reduced $FMNH_2$ [29]. In *P. aeruginosa* UbiX, several strictly conserved residues bind the DMAP phosphate group, with Glu 140 postulated to act as proton donor to enhance the phosphate (or pyrophosphate) leaving group character [8••] (Figures 1, 3a).