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Natural 1,3-Dipolar Cycloadditions

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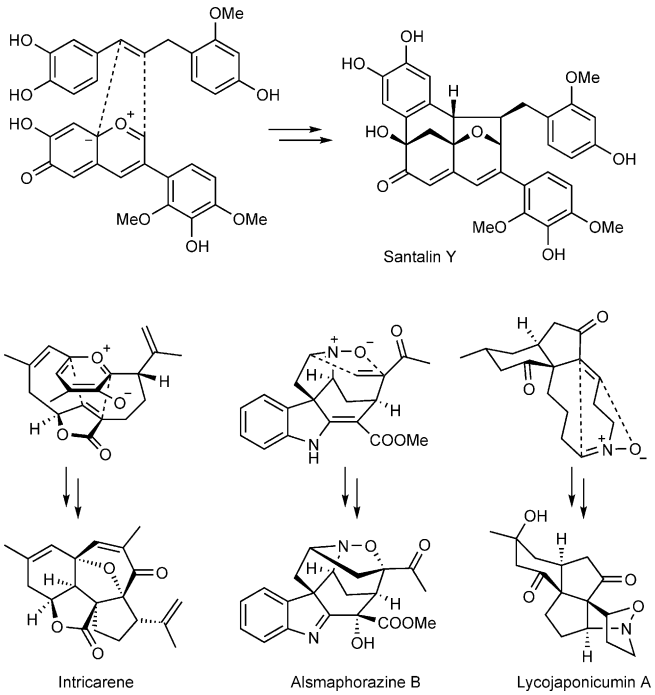
[3+2] cycloaddition · decarboxylation · flavin coenzyme · prenyl transferase · ubiquinone biosynthesis

Enzyme-catalyzed reactions are often a source of inspiration for organic chemists. On the other hand, there are many well-established synthetic protocols for which no enzymatic counterpart has yet been discovered. For decades, much endeavor has been devoted to the search for enzymatic cycloadditions, which culminated in the discovery of true natural and artificial Diels–Alderases.[1] In contrast to the widespread [4+2] cycloadditions in natural product biosynthesis, only little was known about their [3+2] counterparts, which are powerful tools in organic synthesis. Yet the structures of various natural products are suggestive of an involvement of 1,3-dipolar cycloadditions in the corresponding biosynthetic pathways (Scheme 1).[2] Such intriguing models have been supported by the elegant biomimetic total syntheses of santalin Y, intricarene, and alsmaphorazine B, for example.[2a–c] Nonetheless, the involvement of enzymes remained in question since density functional theory (DFT) calculations suggested that nitrone–alkene [3+2] cycloaddition reactions may occur during the biosynthesis of some alkaloid natural products without any enzymatic intervention.[2d] On the other hand, DFT calculations highlighted the impact of hydrogen donors for the proposed 1,3-dipolar intramolecular cycloaddition between a nitrone and an enone in the biosynthesis of lycojaponicumins. A substantial (2000-fold) rate enhancement in a hypothetical active site of an enzyme was inferred.[2e] However, the recent discovery of the first enzyme implicated in a 1,3-dipolar cycloaddition reaction was not structure-guided but fully serendipitous. This rare reaction plays a key role in ubiquinone biosynthesis and detoxification processes in yeast.[3]

Ubiquinone (also known as coenzyme Q, Scheme 2 A) is a terpenoid molecule that is an essential constituent of the electron transport chain. It has long been known that the enzyme couple UbiX and UbiD is involved in the decarboxylation of a ubiquinone precursor.[4] Homologous enzymes from *Saccharomyces cerevisiae*, Pad1 (phenylacrylic acid decarboxylase) and Fdc1 (ferulic acid decarboxylase), play a role in detoxification and the production of volatile compounds (Scheme 2 B).[5] The generally accepted, long-established theory was that these enzyme pairs are isofunctional decarboxylases that independently catalyze the same reaction. However, the precise role of each particular protein has remained a riddle, since various attempts to detect decarboxylase activity in vitro for individual enzymes have failed. Recently, evidence has been unearthed that the two enzymes team up and work in a cooperative fashion.[6] It was found that UbiX and its fungal homologue Pad1 are not responsible for decarboxylation reactions, but instead activate UbiD and Fdc1 by synthesizing a flavine-derived modified cofactor. However, the instability of this cofactor had hampered structure elucidation.[6b] The identity of the enigmatic cofactor has remained a puzzle that dates back to 1976, when it was first observed that the decarboxylation step in ubiquinone biosynthesis requires "an unidentified heat-stable factor of molecular weight less than 10000 [Da]".[7]



Scheme 1. Examples of proposed [3+2] cycloadditions in natural product biosynthesis. Deductions from biomimetic synthesis and DFT calculations.

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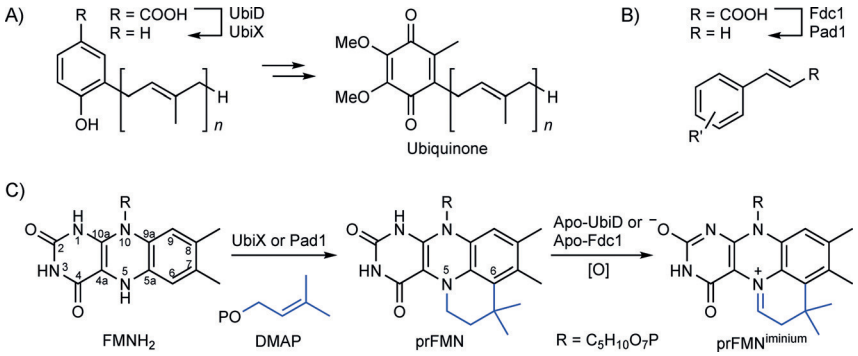


Scheme 2. Enzymatic acid decarboxylation in bacterial ubiquinone biosynthesis (A) and fungal production of off-flavor compounds (B). C) Proposed reaction of DMAP und $FMNH_2$ catalyzed by either UbiX (PDB ID: 4ZAX) or Pad1 to yield aprenylated flavin cofactor (prFMN), which is oxidized into the active form ($prFMN^{iminium}$).

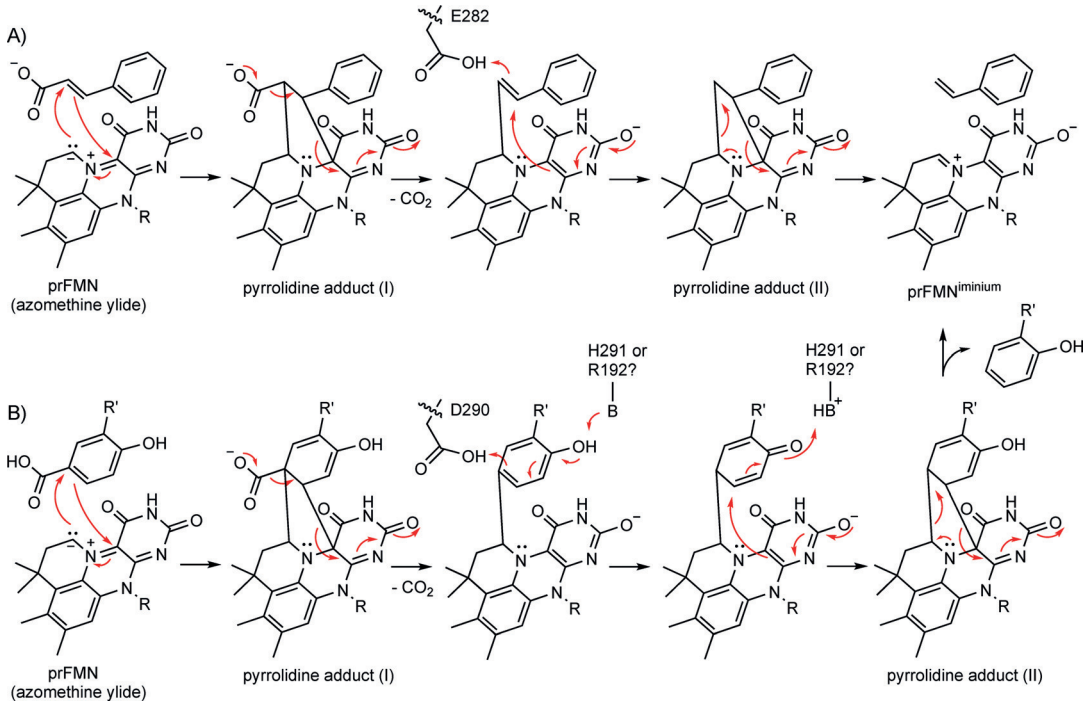
Almost four decades later, the structure of the cofactor has been elucidated by Payne et al. with the help of crystallography and high-resolution structure determination of Fdc1 or, more precisely, $Fdc1^{UbiX}$, which was obtained by coexpressing either *ubiX* or *pad1* with *fdc1* in *E. coli*[3b]. Unlike Fdc1 (produced in the absence of UbiX or Pad1), $Fdc1^{UbiX}$ is able to catalyze the decarboxylation of model substrates in vitro. Distinct spectral characteristics of $Fdc1^{UbiX}$ compared to Fdc1 hinted at a heavily modified flavin mononucleotide (FMN), and eventually, crystal structures of three different variants of $Fdc1^{UbiX}$ revealed that the cofactor is an FMN derivative featuring an additional isoprene-derived ring (prFMN; Scheme 2C).

With this information in hand, the precise role of UbiX in FMN modification was investigated by White et al., but yet another hurdle still had to be overcome: Although UbiX appeared to be a prenyltransferase, dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP), the typical C5 terpene building blocks, were not accepted as substrates. Most surprisingly, spectroscopic evidence for cofactor modification could only be obtained when UbiX was incubated with oxidized FMN and dimethylallyl monophosphate (DMAP). Kinetic crystallography, biochemical studies, spectroscopy, and computational calculations were employed to elucidate the unusual prenylation–cyclization mechanism. According to the current model, the π-electron-rich active-site environment assists in cleaving the phosphate group of DMAP and promotes N5 prenylation. After reorientation of the dimethylallyl moiety, nucleophilic attack by the isoalloxazine group on a transient carbocation yields the additional ring.[3a] UbiX is thus a new member of the growing family of noncanonical prenyl transferases that can also act as cyclases.[8]

This sophisticated mechanism raises the question of why nature makes such huge efforts to alter the structure of a flavin cofactor. Flavin coenzymes are versatile heterocycles that enable enzymes to catalyze an enormous range of redox transformations in biosynthetic pathways. The catalytic repertoire of these cofactors includes both two- and one-electron transfers that allow flavoenzymes to facilitate substrate oxidations and reductions.[9] Typically, N5 of the FMN isoalloxazine ring has a very special role as a site of hydride addition or ejection, as well as transient covalent addition of substrate carbanions. N5-alkylation of FMN thus impedes conventional flavin catalysis, which is very surprising. But as is so often the case, when one door closes, another one opens: when oxidized to the corresponding flavo-N5 iminium adduct ($prFMN^{iminium}$), prFMN is easily transformed into an azomethine ylide (Scheme 3). This well-known 1,3-dipole can react with diverse dipolarophiles in [3+2] cycloadditions. From in vitro decarboxylation experiments using several cinnamic acid derivatives and from structural data, a reaction mechanism was deduced in which the decarboxylation is promoted by a transient covalent pyrrolidine adduct.[3] The styrene product would be released after the formation of another pyrrolidine adduct, and the remaining $prFMN^{iminium}$ would then be transformed back into the azomethine ylide (Scheme 3).[3b] Although no direct evidence for a concerted reaction was given, this is a plausible scheme for an enzyme-catalyzed biological equivalent of a [3+2] cycloaddition.

Whereas this model explains how styrenes and other off-flavor compounds are produced during *S. cerevisiae* fermentations, the mechanism for the decarboxylation of the bacterial ubiquinone precursor, which clearly differs from the cinnamate dipolarophile, has not yet been investigated. However, from synthetic studies it is known that 1,3-dipolar cycloadditions involving azomethine ylides may also involve aromatic compounds, although aromaticity is lost in intermediary states. For the [3+2] cycloaddition with aromatic dipolarophiles, it is favorable to use benzenes that are directly tethered to the ylide, embedded in polycyclic aromatic systems, or substituted with highly electron-withdrawing moieties such as nitro groups.[10] It is conceivable that the active site of UbiD provides the electronic and entropic prerequisites for [3+2] cycloaddition of the *p*-hydroxy benzoic acid derivative and prFMN azomethine ylide. In fact, we noted that the active site of UbiD harbors basic residues that might support the proposed reaction sequence (Scheme 3B; also see the Supporting Information).

In conclusion, biomimetic studies and biosynthetic considerations have strongly suggested that [3+2] cycloadditions occur in nature. However, the first example of a biocatalytic 1,3-dipolar cycloaddition was discovered during the investigation of an apparently simple enzymatic decarboxylation reaction. Altogether these findings will stimulate the search



Scheme 3. A) Proposed mechanism for the decarboxylation reaction of the model substrate cinnamic acid to give styrene, as catalyzed by Fdc1 (PDB ID: 4A7). A transient pyrrolidine adduct forms in a 1,3 dipolar cycloaddition of the azomethine ylide resonance form of prFMN and cinnamic acid. B) A model for aromatic acid decarboxylation by UbiD (PDB ID: 2IDB).

for related reactions in biosynthetic pathways and the development of biomimetic synthetic reactions.

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