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Review

Biosynthesis of vitamin B_2: Structure and mechanism of riboflavin synthase

Markus Fischer [a,*], Adelbert Bacher [b]

[a] *Institute of Food Chemistry, University of Hamburg, Grindelallee 117, D-20146 Hamburg, Germany*
[b] *Lehrstuhl für Biochemie, Technische Universität München, Lichtenbergstr. 4, D-85747 Garching, Germany*

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Abstract

The biosynthesis of one riboflavin molecule requires one molecule of GTP and two molecules of ribulose 5-phosphate as substrates. GTP is hydrolytically opened, converted into 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione by a sequence of deamination, side chain reduction and dephosphorylation. Condensation with 3,4-dihydroxy-2-butanone 4-phosphate obtained from ribulose 5-phosphate leads to 6,7-dimethyl-8-ribityllumazine. The final step in the biosynthesis of the vitamin involves the dismutation of 6,7-dimethyl-8-ribityllumazine catalyzed by riboflavin synthase. The mechanistically unusual reaction involves the transfer of a four-carbon fragment between two identical substrate molecules. The second product, 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione, is recycled in the biosynthetic pathway by 6,7-dimethyl-8-ribityllumazine synthase. This article will review structures and reaction mechanisms of riboflavin synthases and related proteins up to 2007 and 122 references are cited.

Keywords: Vitamin B_2; Riboflavin synthase; Lumazine synthase; Lumazine protein; X-ray structure; Reaction mechanism

In association with a wide variety of apoenzymes, the flavocoenzymes derived from vitamin B_2 (riboflavin) are probably the most chemically versatile cofactors. They have long been known to enable a wide variety of redox reactions involving one- as well as two-electron transfer processes, whereas other coenzymes are typically limited to one of them (for example, pyridine nucleotides catalyze exclusively two-electron transfers) [1,2]. Moreover, they are involved in an impressive number of reactions that do not involve a net exchange of electrons with the respective substrate. For a comprehensive description of flavoenzymes that catalyze reactions with no net redox change the reader is directed to the review by Bornemann [3].

Flavins serve as primary and secondary emitters in bacterial luminescence. Lumazine proteins, which can bind riboflavin, FMN, 6,7-dimethyl-8-ribityllumazine or 6-methyl-7-oxo-8-ribityllumazine, are believed to act as optical transponders in the bioluminescence emission of photobacteria [4–8]. The interposition of lumazine protein as an optical transponder between the energy generation and light emission steps has been proposed to result in shifts of the emission maximum and an increased quantum yield [9].

This extraordinary photochemical functionality reflects the enormous versatility of the isoalloxazine chromophore, whereas the side chains of the flavocoenzymes serve primarily as anchors that secure their binding to the cognate apoproteins, which typically form relatively tight complexes. Moreover, flavocoenzymes can form very complex catalytic sites involving more than one flavocoenzyme, modified flavins and/or additional cofactors such as iron sulfur clusters [10–13].

In some cases, flavocoenzymes become covalently linked to their apoenzymes [14]. Photoreceptors involved in processes like stem bending toward a light source (phototropism), chloroplast migration to places of appropriate light intensity (chloroplast photorelocation), and the

* Corresponding author. Fax: +49 40428384342.
E-mail address: markus.fischer@uni-hamburg.de (M. Fischer).

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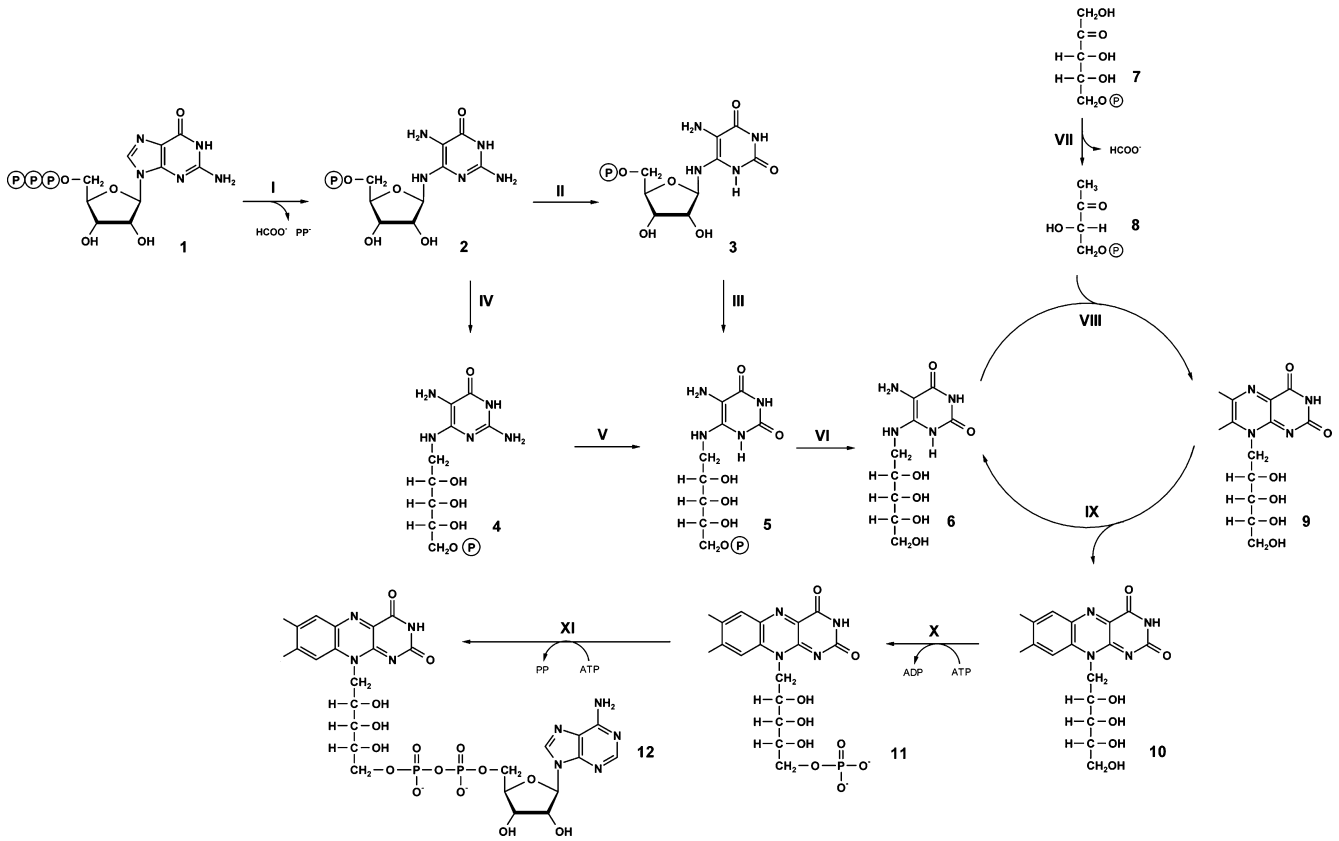

Fig. 1. Biosynthesis of riboflavin and flavocoenzymes. Step I, GTP cyclohydrolase II; step II, 2,5-diamino-6-ribosylamino-4(3*H*)-pyrimidinone 5′-phosphate deaminase; step III, 5-amino-6-ribosylamino-2,4(1*H*,3*H*)-pyrimidinedione 5′-phosphate reductase; step IV, 2,5-diamino-6-ribosylamino-4(3*H*)-pyrimidinone 5′-phosphate reductase; step V, 2,5-diamino-6-ribitylamino-4(3*H*)-pyrimidinone 5′-phosphate deaminase; step VI, hypothetical phosphatase; step VII, 3,4-dihydroxy-2-butanone 4-phosphate synthase; step VIII, 6,7-dimethyl-8-ribityllumazine synthase; step IX, riboflavin synthase; step X, riboflavin kinase; step XI, FAD synthetase; **1**, GTP; **2**, 2,5-diamino-6-ribosylamino-4(3*H*)-pyrimidinone 5′-phosphate; **3**, 5-amino-6-ribosylamino-2,4(1*H*,3*H*)-pyrimidinedione 5′-phosphate; **4**, 2,5-diamino-6-ribitylamino-4(3*H*)-pyrimidinone 5′-phosphate; **5**, 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione 5′-phosphate; **6**, 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione; **7**, ribulose 5′-phosphate; **8**, 3,4-dihydroxy-2-butanone 4-phosphate; **9**, 6,7-dimethyl-8-ribityllumazine; **10**, riboflavin; **11**, FMN; **12**, FAD.

opening of stomatal guard cells to facilitate gas exchange are the plasma membrane-associated phototropins and homologs [15–21]. The common features of the phototropin photoreceptor family are two light-sensitive domains, LOV1 and LOV2, and a serine/threonine kinase domain. Each of the two LOV domains non-covalently binds a single flavin mononucleotide (FMN)[1] as a chromophore. After illumination by blue light, the LOV photocycle comprises a light-induced addition of a thiol group to the C(4a) position of the FMN chromophore [20,22].

Flavins have also been shown to be involved in circadian and seasonal clocks [23–25].

The mechanistic complexity of flavoprotein catalysis is to some extent matched by the complexity of some of the reactions involved in the biosynthesis of the isoalloxazine moiety of riboflavin. Surprisingly, some of these mechanistically complex reactions can proceed without catalysis under relatively mild conditions [26–33]. Thus, it is conceivable that the formation of flavin could initially have occurred by spontaneous processes, prior to the evolution of macromolecular biocatalysis.

The biosynthesis of riboflavin has been reviewed repeatedly and the reader is directed to these articles for an overview of the pathway (Fig. 1) [34–38]. This article will focus on the last step in the biosynthetic pathway, which continues to present fascinating mechanistic problems even after more than four decades of research.

Briefly, the initial steps of the riboflavin pathway involve the conversion of GTP (**1**) into 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione (**6**). The intermediate is obtained via structurally different precursors in different organisms. More specifically, the first committed pathway intermediate, 2,5-diamino-6-ribosylamino-4(3*H*)-pyrimidinone 5′-phosphate (**2**), is reduced and then deaminated in fungi and in Archaea [39,40], whereas reduction of the side chain precedes deamination in eubacteria and plants [41–44]. It is still unknown how the phosphate residue in 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione 5′-phosphate (**5**) is released to generate 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione (**6**). It is clear, however,

[1] *Abbreviations used:* FMN, flavin mononucleotide; MjaRS, *M. jannaschii*.