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Cofactor biosynthesis—still yielding fascinating new biological chemistry

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Abstract

This mini review covers recent advances in the mechanistic enzymology of cofactor biosynthesis.

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Introduction

Cofactor biosynthetic pathways use a larger amount of novel organic chemistry than any of the other pathways in primary metabolism, and mechanistic studies on cofactor biosynthetic enzymes continue to be a rich area of investigation. In this perspective, we will describe recent advances in the biosynthesis of thiamin, molybdopterin, pyridoxal phosphate, nicotinamide adenine dinucleotide, heme, and vitamin B_{12}, with a focus on reactions that pose novel mechanistic problems.

Thiamin biosynthesis in bacteria

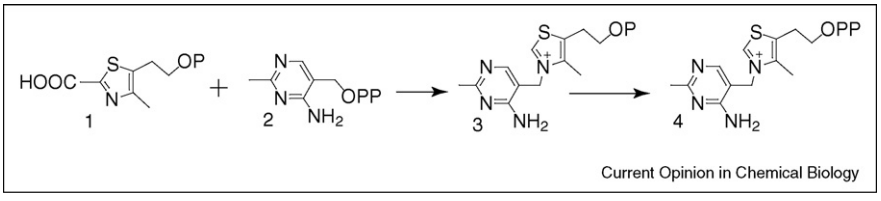
The bacterial thiamin biosynthetic pathway involves the separate biosynthesis of thiazole 1 and pyrimidine 2. These are then coupled to give thiamin phosphate 3. The mechanism of the coupling enzyme is now well understood: the intrinsic rate of carbocation formation has been determined and the pyrimidine carbocation intermediate has been structurally characterized [1•] (Figure 1).

The current mechanistic proposal for thiazole formation is shown in Figure 2. DXP 5 forms an imine with Lys96 of the thiazole synthase (ThiG) to give 6. Tautomerization followed by thiocarboxylate addition to the C3 ketone of 7 gives 9, which then undergoes an S/O acyl shift followed by water loss to give thioketone 11. Tautomerization followed by elimination of the sulfur carrier protein (ThiS, 15) gives 13. Addition of the glycine imine 14, formed in a separate reaction, followed by a transimination generates the thiazole tautomer 17. A separate enzyme (TenI) is required to catalyze the aromatization of 17 to 1. This proposal is supported by product characterization, substrate analog studies, the trapping of the imine 6, the demonstration of enzyme-catalyzed H/D exchange at C3 of DXP, the demonstration that a carboxy terminal oxygen of ThiS 15 is derived from DXP 5, the trapping of 13, and the structures of the ThiSG and ThiFS complexes [2•,3•,4•,5•,6•,7•,8•].

In most bacteria, glycine oxidation to give 14 is catalyzed by an oxygen-requiring flavoenzyme (ThiO) [9•]. In *Escherichia coli* and some other proteobacteria, in which thiamin biosynthesis can occur under anaerobic conditions, the glycine imine is formed from tyrosine in a reaction catalyzed by ThiH, a radical SAM enzyme. A mechanistic proposal is outlined in Figure 3. Hydrogen atom abstraction from tyrosine 18 gives the phenoxy radical 20, which can then undergo fragmentation to 22 and 23. A second electron transfer gives the glycine imine 14 [10••].

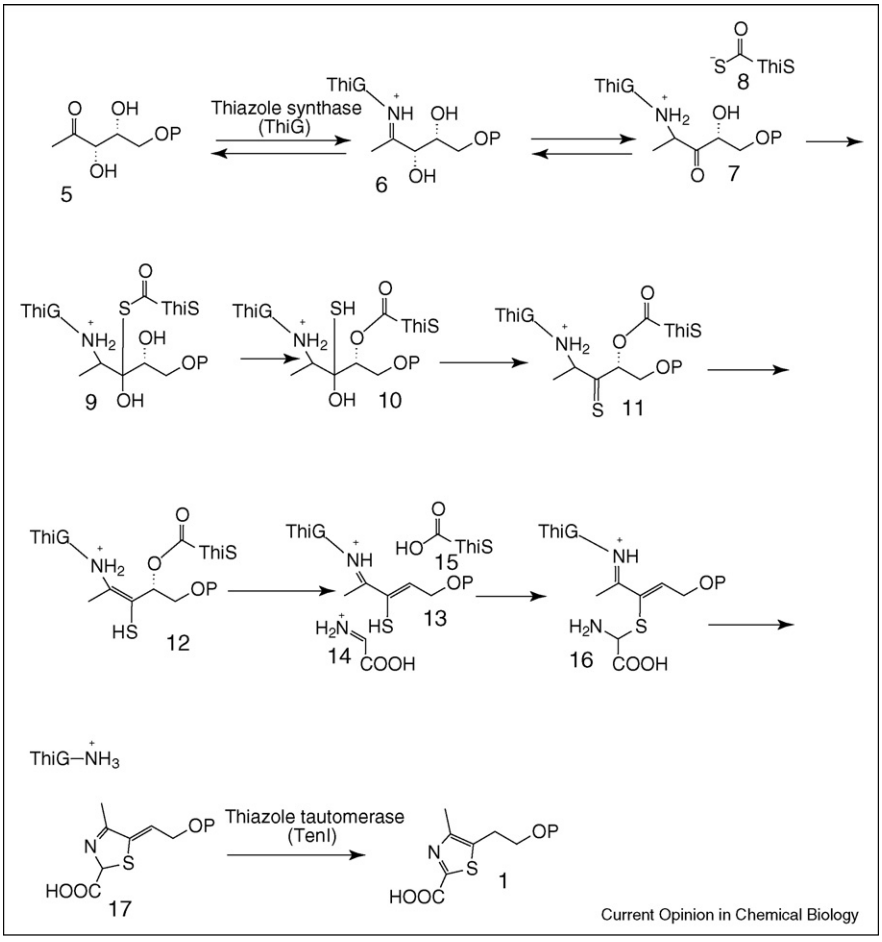
The biosynthesis of the pyrimidine moiety of thiamin occurs by a remarkable rearrangement of aminoimidazole ribotide catalyzed by the ThiC gene product. The results

Figure 1



Thiamin phosphate 3 is biosynthesized by coupling the thiazole 1 with the pyrimidine 2.

Figure 2



Mechanistic proposal for the formation of the thiamin thiazole 1 in bacteria. ThiS represents the sulfide carrier protein.

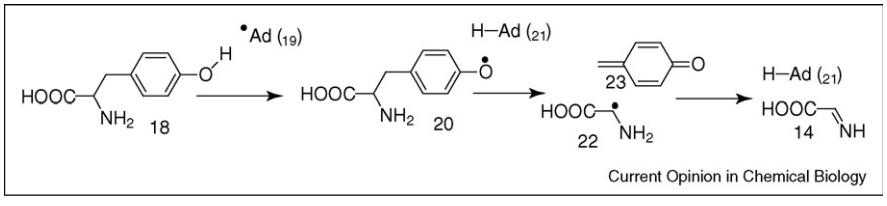
of a comprehensive labeling study are shown in Figure 4 [11••]. This reaction has now been reconstituted in a defined biochemical system. The pyrimidine synthase activity was found to be dependent on a functional iron–sulfur cluster and on *S*-adenosyl methionine (SAM). Production of 5-deoxyadenosine from SAM during pyrimidine formation suggests that this enzyme belongs to the radical SAM superfamily of enzymes. However, the mechanism of the rearrangement reaction has not yet been elucidated.

Thiamin biosynthesis in *Saccharomyces cerevisiae*

While the later steps in thiamin biosynthesis in *Saccharomyces cerevisiae* are similar to those in bacteria, the biosynthesis of the two heterocycles is different.

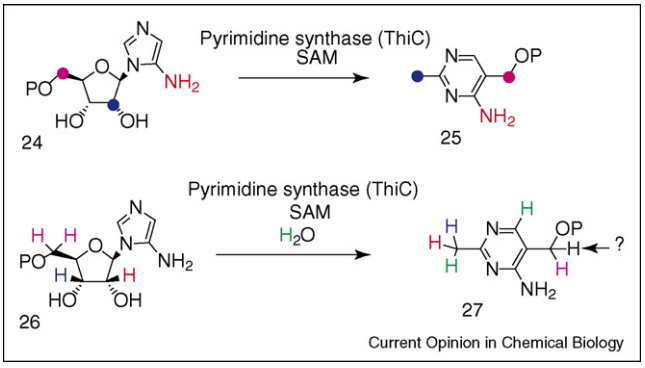
Considerable progress has been made with the enzymology of thiazole formation. While the reaction catalyzed by the thiazole synthase (THI4) has not yet been fully reconstituted, the enzyme co-purified with three tightly bound metabolites (35, 40, and 41) and the structure of the enzyme

Figure 3



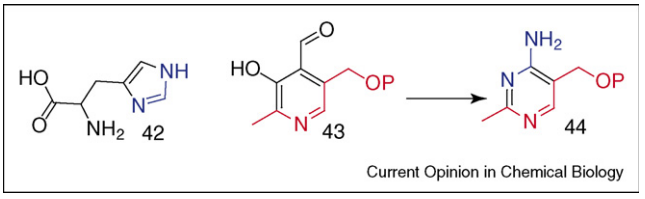
Mechanistic proposal for the ThiH-catalyzed formation of glycine imine (14) under anaerobic conditions.

Figure 4



Labeling studies that map out the complex conversion of aminoimidazole ribotide to the thiamin pyrimidine.

Figure 6



The pyrimidine moiety of thiamin in *S. cerevisiae* is formed by a remarkable reaction sequence in which the histidine atoms labeled in blue and the PLP atoms labeled in red are incorporated into the pyrimidine 44.

complexed to 41 has been determined [12••, 13•,14•,15•]. The identity of these metabolites suggested that NAD is the precursor to the thiazole. This was confirmed by the identification of three partial reactions catalyzed by the C204A mutant of THI4 (28 to 29, 29 to 30 and 29 to 35 in Figure 5) [12••]. Combining this information suggests the mechanism of thiazole formation outlined in Figure 5.

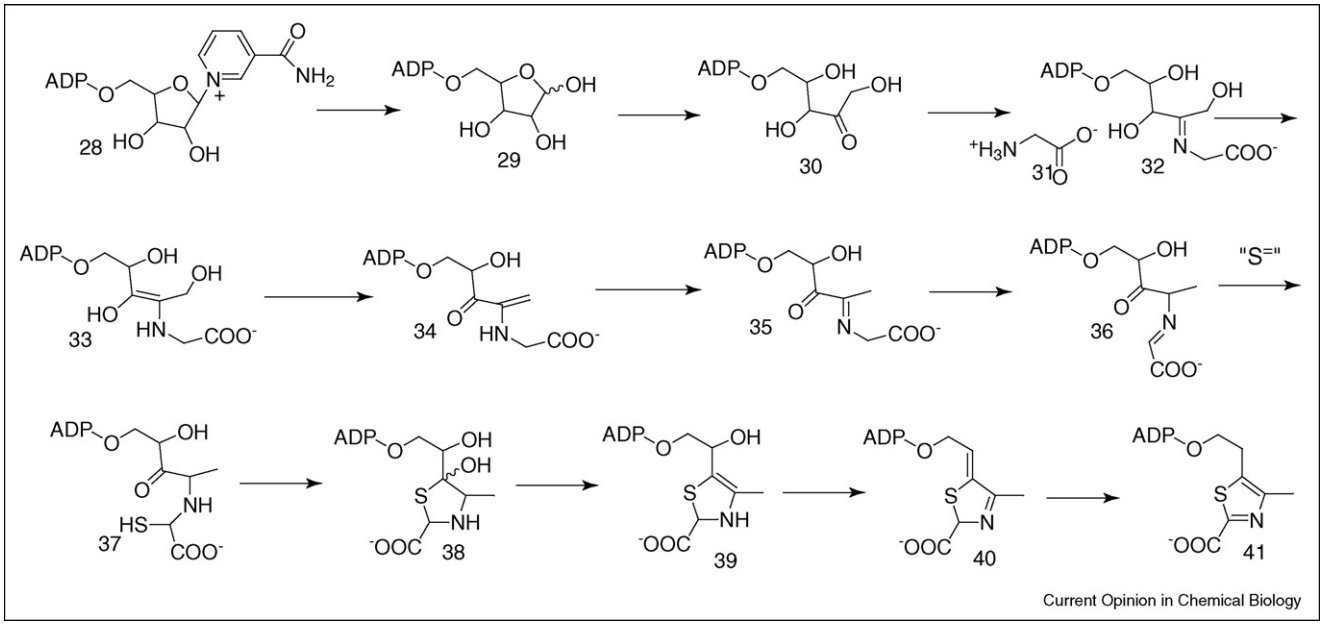
In this mechanism, cleavage of the *N*-glycosyl bond of NAD 28 followed by ring opening and tautomerization gives 30. Imine formation with glycine 31, tautomerization and water loss gives 34. Two tautomerizations followed by sulfide addition gives 37, which then cyclizes to 38. Loss of two molecules of water to give 40 followed by thiazole aromatization completes the reaction. The sulfur source depicted in the conversion of 36 to 37 has not yet been unambiguously identified.

The thiamin pyrimidine in *S. cerevisiae* is derived from histidine 42 and PLP 43 in a reaction catalyzed by the THI5 gene product (Figure 6) [16••]. The enzymology of this remarkable process is not yet understood.

Thiamin salvage

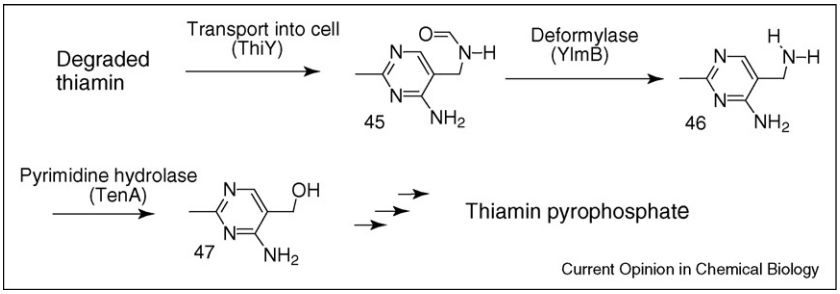
A new pathway for the salvage of the thiamin pyrimidine from thiazole-degraded thiamin has recently been discovered (Figure 7). In this pathway, thiazolium-degraded forms of thiamin (e.g. 45) bind to the periplasmic component of an ABC transporter (ThiY) and are then

Figure 5



Mechanistic proposal for the formation of the thiamin thiazole (41) in *S. cerevisiae*.

Figure 7



A new thiamin salvage pathway for the recycling of the pyrimidine of thiazole-degraded thiamin.

transported into the cell. Deformylation to 46 followed by hydrolysis gives 47, which can then be incorporated into the thiamin biosynthetic pathway. This pathway is widely distributed in bacteria, archaea, and eukaryotes [17••].

Molybdopterin biosynthesis

The enzyme catalyzing the formation of Precursor Z (49) from GTP (48) is a radical SAM enzyme (Figure 8). The structure of this enzyme has been determined, but the reaction mechanism is still unknown [18•].

Pyridoxal phosphate (PLP) biosynthesis

Just at the point when we thought PLP biosynthesis was a solved problem [19••], an entirely new pathway emerged from studies on singlet oxygen resistance in *Cercospora nicotianae* [20•]. This new pathway has now been reconstituted using ribose-5-phosphate glutamine and glyceraldehyde-3-phosphate as substrates, and the new PLP synthase has been structurally and mechanistically characterized [21•,22••,23••,24•]. A mechanistic proposal is outlined in Figure 9.

In this mechanism, ribose-5-phosphate 50 undergoes ring opening and forms an imine 52 with an active site lysine. Tautomerization followed by ammonia addition gives 54. Loss of water followed by a tautomerization gives 56. Elimination of lysine from C1 followed by its addition to C5 gives 58. Loss of phosphate from 58 gives 59. Addition of glyceraldehyde-3-phosphate 60 gives imine 61. A double tautomerization to 63 followed by an electrocyclic ring closure to 64 and dehydration gives 65. Imine hydrolysis completes the reaction [25••,26•].

Nicotinamide adenine dinucleotide (NAD) biosynthesis

The formation of quinolinic acid 68, the precursor to the pyridine ring of NAD 28, is a long-standing unsolved problem in biosynthesis. Two routes have been identified: one involving the condensation of aspartic acid imine 66 with dihydroxyacetone phosphate 67, the other involving the oxidation of hydroxyanthranilate 69.

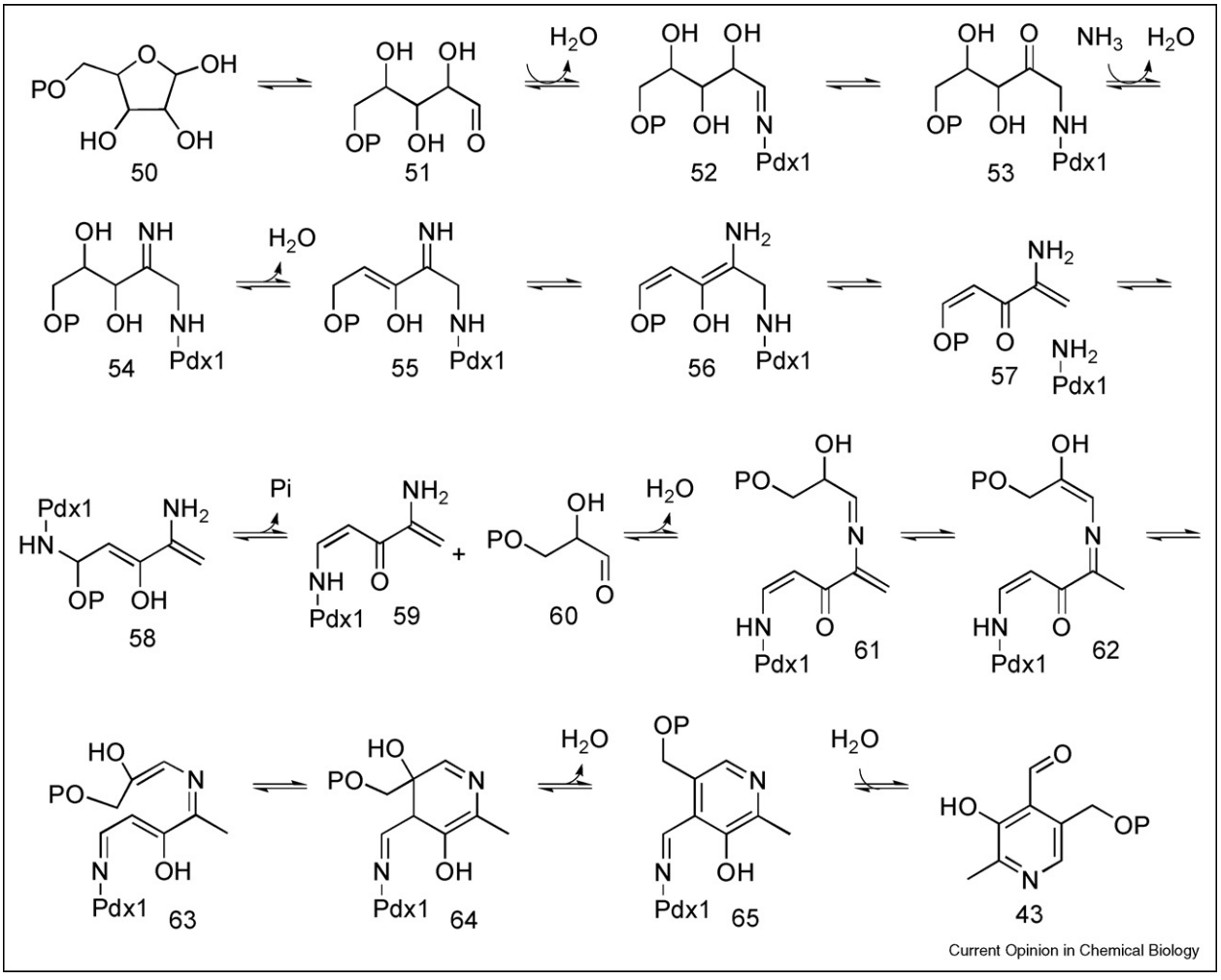
The condensation of the imine of aspartic acid 66 with dihydroxyacetone phosphate 67 to form quinolinic acid 68 has only recently been reconstituted using purified enzyme. This enzyme contains an iron sulfur cluster, and a structure of the apoenzyme has been solved [27•,28•]. No mechanistic studies have yet been reported (Figure 10).

The mechanism for the oxidation of hydroxyanthranilate 69 to 2-amino-3-carboxymuconic acid semialdehyde 70 is now relatively well understood and proceeds by an extradiol dioxygenase type mechanism [29••]. Compound 70 then undergoes a nonenzymatic tautomerization/isomerization/electrocyclization sequence followed by a dehy-

Figure 8

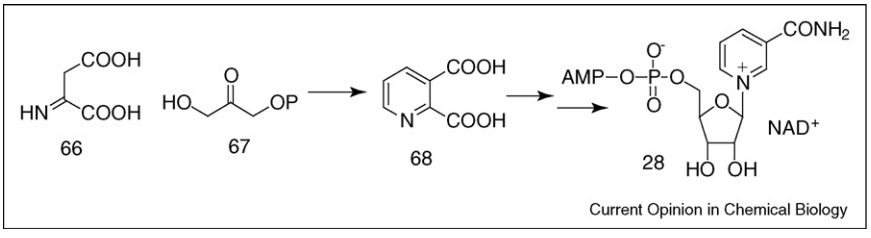
MoaA and MoaC catalyze the formation of Precursor Z (49) on the molybdopterin biosynthetic pathway.

Figure 9



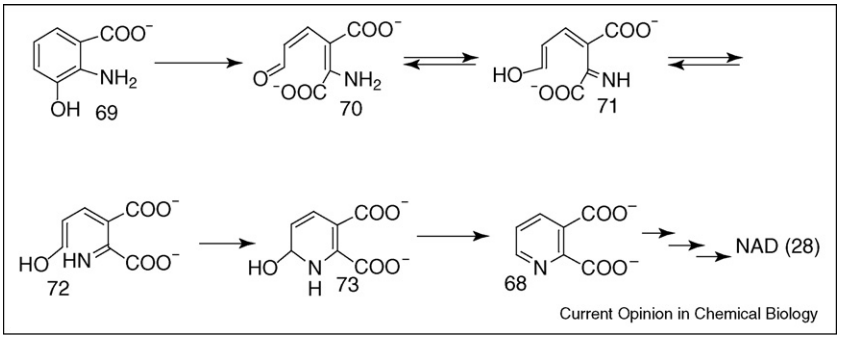
A mechanistic proposal for the formation of PLP (43). Pdx1 represents the pyridoxal phosphate synthase.

Figure 10



The major bacterial pathway for the formation of the quinolinic acid (68) precursor to NAD 28 is shown.

Figure 11



The mechanistic proposal for the formation of quinolinic acid 68 from hydroxyanthranilate (69) is shown.

dration to form quinolinic acid as outlined in Figure 11 [30•].

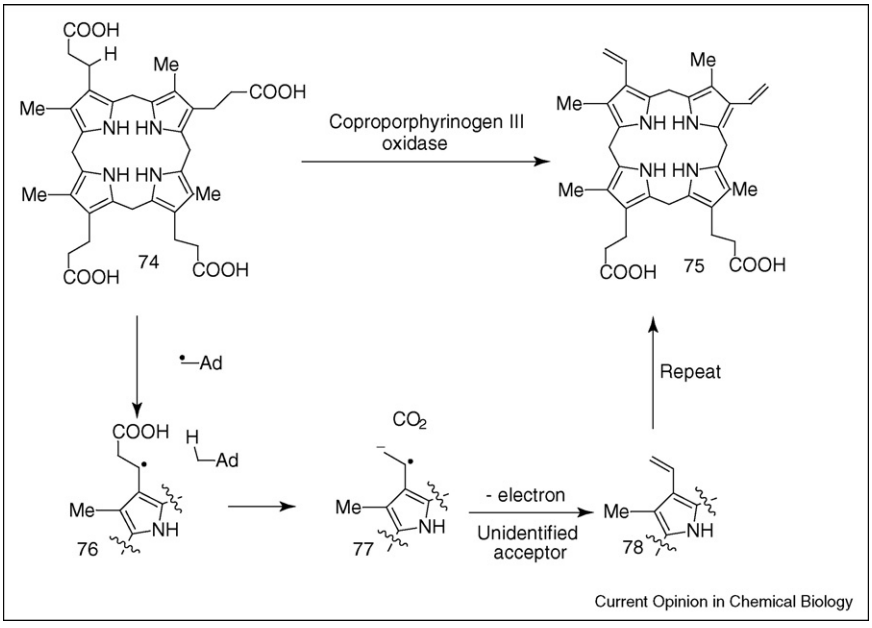
Porphyrin biosynthesis

The characterization of the oxygen-independent coproporphyrinogen III oxidase (HemN) is an exciting recent advance in porphyrin biosynthesis. This enzyme catalyzes the reaction shown in Figure 12 and is a radical SAM enzyme. The structure of this enzyme has been solved, and a substrate-derived radical was characterized by ESR spectroscopy [31•,32••]. The reaction is likely to proceed by hydrogen atom abstraction to give 76 followed by decarboxylation and a second electron transfer to give 78. Repetition of this sequence would give 75.

Vitamin B_{12}

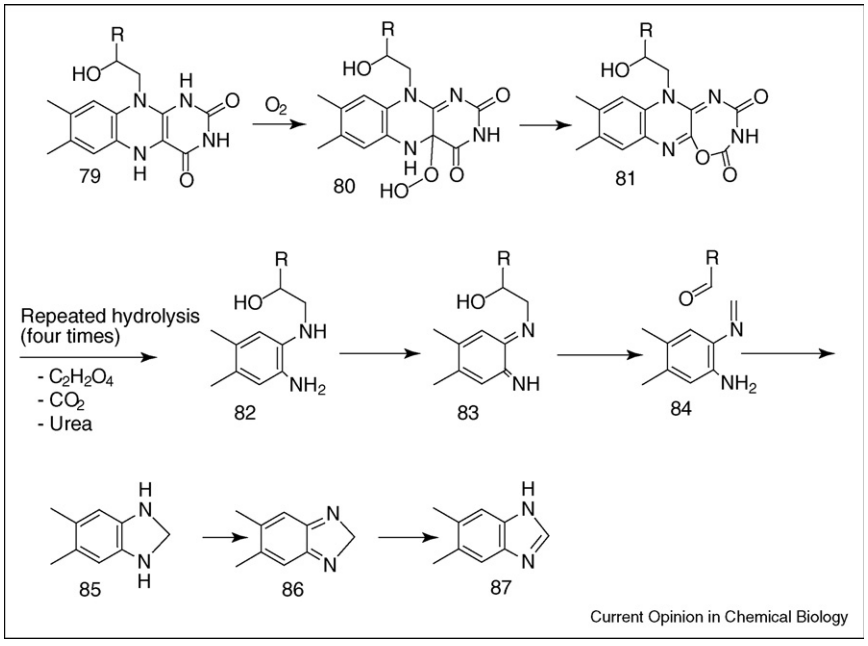
The dimethylbenzimidazole ligand 87 of vitamin B_{12} is formed from flavin mononucleotide (FMN). The enzyme catalyzing this remarkable transformation has now been identified, and an early intermediate in which molecular oxygen is poised for attack on the flavin has been structurally characterized [33•]. A mechanistic proposal, consistent with the structure, previous labeling studies, and model chemistry is outlined in Figure 13 [34]. In this mechanism, reduced FMN 79 reacts with molecular oxygen to form the hydroperoxide 80. A Baeyer-Villiger like rearrangement to 81 followed by four hydrolysis reactions gives 82. A 2-electron oxidation of 82 to bisimine 83, a retroaldol fragmentation to 84, and a cyclization would give 85. A final 2-electron oxidation followed

Figure 12



Mechanistic proposal for the oxidative decarboxylation of coproporphyrinogen III (74) to protoporphyrinogen IX (75).

Figure 13



Mechanistic proposal for the oxidation of FMN 79 to dimethylbenzimidazole 87.

by a tautomerization would complete the formation of the DMB ligand.

Conclusions

While our knowledge of cofactor biosynthetic pathways is now at an advanced stage, the mechanistic chemistry of several of the reactions involved remains to be elucidated. These reactions include the formation of the pyrimidine moiety of thiamin in both prokaryotes and eukaryotes, the formation of Precursor Z on the molybdopterin pathway, the formation of quinolinic acid in bacteria, and the formation of the vitamin B_{12} DMB ligand. It is also likely that new pathways to some of the cofactors remain to be discovered in the wealth of information now available from genome sequences and environmental DNA libraries. In addition to the discovery of new biosynthetic chemistry, our increasing understanding of cofactor biosynthetic chemistry will facilitate the production of vitamins by fermentation and the exploration of cofactor biosynthetic enzymes as antibiotic targets.

References and recommended reading

Papers of particular interest, published within the annual period of the review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hanes JW, Ealick SE, Begley TP: **Thiamin phosphate synthase:**
• **the rate of pyrimidine carbocation formation**. *J Am Chem Soc* 2007, **129**:4860-4861.

Structural and kinetic characterization of the pyrimidine carbocation intermediate generated at the active site of thiamin phosphate synthase.

2. Chatterjee A, Han X, McLafferty FW, Begley TP: **Biosynthesis of**
• **thiamin thiazole: determination of the regiochemistry of the S/O acyl shift by using 1,4-dideoxy-D-xylulose-5-phosphate**. *Angew Chem Int Ed* 2006, **45**:3507-3510.

Determination that the acyl shift in thiamin thiazole biosynthesis occurs to the C3 hydroxyl of DXP.

3. Dorrestein PC, Zhai H, McLafferty FW, Begley TP: **The**
• **biosynthesis of the thiazole phosphate moiety of thiamin: the sulfur transfer mediated by the sulfur carrier protein ThiS**. *Chem Biol* 2004, **11**:1373-1381.

Mechanistic characterization of the bacterial thiazole synthase.

4. Dorrestein PC, Zhai H, Taylor SV, McLafferty FW, Begley TP: **The**
• **biosynthesis of the thiazole phosphate moiety of thiamin (vitamin B1): the early steps catalyzed by thiazole synthase**. *J Am Chem Soc* 2004, **126**:3091-3096.

Mechanistic characterization of the early steps in bacterial thiazole biosynthesis.

5. Duda DM, Walden H, Sfondouris J, Schulman BA: **Structural**
• **analysis of *Escherichia coli* ThiF**. *J Mol Biol* 2005, **349**: 774-786.

The structure of the enzyme involved in the activation of the sulfur carrier protein involved in thiazole biosynthesis.

6. Lehmann C, Begley TP, Ealick SE: **Structure of the *Escherichia***
• ***coli* ThiS–ThiFcomplex, a key component of the sulfur transfer system in thiamin biosynthesis**. *Biochemistry* 2006, **45**:11-19.

The structure of the ThiF–ThiS complex involved in sulfur transfer from cysteine to the thiamin thiazole.

7. Park J-H, Dorrestein PC, Zhai H, Kinsland C, McLafferty FW,
• Begley TP: **Biosynthesis of the thiazole moiety of thiamin pyrophosphate (vitamin B1)**. *Biochemistry* 2003, **42**:12430-12438.

Reconstitution of bacterial thiazole biosynthesis in a biochemically defined system.

8. Settembre EC, Dorrestein PC, Zhai H, Chatterjee A,
• McLafferty FW, Begley TP, Ealick SE: **Thiamin biosynthesis in *Bacillus subtilis*: structure of the thiazole synthase/sulfur carrier protein complex**. *Biochemistry* 2004, **43**: 11647-11657.

Structural characterization of the bacterial thiazole synthase reveals a simple active site catalyzing a complex reaction.

9. Settembre EC, Dorrestein PC, Park J-H, Augustine AM, Begley TP,
• Ealick SE: **Structural and mechanistic studies on ThiO, a glycine oxidase essential for thiamin biosynthesis in *Bacillus subtilis***. *Biochemistry* 2003, **42**:2971-2981.
Structural studies on ThiO support a hydride transfer mechanism.

10. Kriek M, Martins F, Leonardi R, Fairhurst SA, Lowe DJ, Roach PL:
•• **Thiazole synthase from *Escherichia coli*: an investigation of the substates and purified proteins required for activity *in vitro***. *J Biol Chem* 2007, **282**:17413-17423.
Reconstitution of the ThiH-dependent thiazole biosynthesis and the identification that ThiH is a radical SAM enzyme.

11. Lawhorn BG, Mehl RA, Begley TP: **Biosynthesis of the thiamin
•• pyrimidine: the reconstitution of a remarkable rearrangement reaction**. *Org Biomol Chem* 2004, **2**:2538-2546.
Reconstitution of the remarkable conversion of 5-aminoimidazole ribonucleotide to the thiamin pyrimidine.

12. Chatterjee A, Jurgenson CT, Schroeder FC, Ealick SE, Begley TP:
•• **Biosynthesis of thiamin thiazole in eukaryotes: conversion of NAD to an advanced intermediate**. *J Am Chem Soc* 2007, **129**:2914-2922.
Demonstration that the thiamin thiazole in eukaryotes is derived from NAD.

13. Jurgenson CT, Chatterjee A, Begley TP, Ealick SE: **Structural
• insights into the function of the thiamin biosynthetic enzyme Thi4 from *Saccharomyces cerevisiae***. *Biochemistry* 2006, **45**:11061-11070.
Structural studies on the eukaryotic thiazole synthase from *Saccharomyces cerevisiae* showing bound ADP-thiazole.

14. Chatterjee A, Jurgenson CT, Schroeder FC, Ealick SE, Begley TP:
• **Thiamin biosynthesis in eukaryotes: characterization of the enzyme-bound product of thiazole synthase from *Saccharomyces cerevisiae* and its implications in thiazole biosynthesis**. *J Am Chem Soc* 2006, **128**:7158-7159.
The identification of a Thi4-bound metabolite reveals the function of the eukaryotic thiazole synthase.

15. Godoi PHC, Galhardo RS, Luche DD, Van Sluys M-A, Menck CFM,
• Oliva G: **Structure of the thiazole biosynthetic enzyme THI1 from *Arabidopsis thaliana***. *J Biol Chem* 2006, **281**:30957-30966.
Structural studies on the eukaryotic thiazole synthase from *Arabidopsis thaliana* showing bound ADP-thiazole.

16. Zeidler J, Sayer BG, Spenser ID: **Biosynthesis of vitamin B1 in
•• yeast. Derivation of the pyrimidine unit from pyridoxine and histidine. Intermediacy of urocanic acid**. *J Am Chem Soc* 2003, **125**:13094-13105.
Isotopic labeling studies demonstrate that the pyrimidine moiety of thiamin in *S. cerevisiae* is derived from histidine and PLP.

17. Jenkins AH, Schyns G, Potot S, Sun G, Begley TP: **A new thiamin
•• salvage pathway**. *Nat Chem Biol* 2007, **3**:492-497.
Functional studies on TenA lead to a new thiamin salvage pathway.

18. Hanzelmann P, Schindelin H: **Binding of 5′-GTP to the C-
• terminal FeS cluster of the radical *S*-adenosylmethionine enzyme MoaA provides insights into its mechanism**. *Proc Natl Acad Sci* 2006, **103**:6829-6834.
Structural studies on MoaA, an intriguing enzyme catalyzing a new rearrangement of GTP.

19. Cane DE: **Mechanism and structure of biosynthetic enzymes.
•• The biosynthesis of vitamin B6**. *Chimia* 2003, **57**:75-76.
A review describing the current status of our understanding of the *E. coli* PLP biosynthetic pathway.

20. Ehrenshaft M, Bilski P, Li MY, Chignell CF, Daub ME: **A highly
• conserved sequence is a novel gene involved in de novo vitamin B6 biosynthesis**. *Proc Natl Acad Sci* 1999, **96**:9374-9378.
A major contribution pointing toward a new PLP biosynthetic pathway based on singlet oxygen resistance.

21. Raschle T, Arigoni D, Brunisholz R, Rechsteiner H, Amrhein N,
• Fitzpatrick TB: **Reaction mechanism of pyridoxal 5′-phosphate synthase: detection of an enzyme-bound chromophoric intermediate**. *J Biol Chem* 2007, **282**:6098-6105.
Mechanistic analysis of the DXP-independent PLP synthase.

22. Strohmeier M, Raschle T, Mazurkiewicz J, Rippe K, Sinning I,
•• Fitzpatrick TB, Tews I: **Structure of a bacterial pyridoxal 5′-phosphate synthase complex**. *Proc Natl Acad Sci U S A* 2006, **103**:19284-19289.
Structural studies on the DXP-independent PLP synthase.

23. Zein F, Zhang Y, Kang Y-N, Burns K, Begley TP, Ealick SE:
•• **Structural insights into the mechanism of the PLP synthase holoenzyme from *Thermotoga maritima***. *Biochemistry* 2006, **45**:14609-14620.
Structural studies on the DXP-independent PLP synthase.

24. Burns KE, Xiang Y, Kinsland CL, McLafferty FW, Begley TP:
• **Reconstitution and biochemical characterization of a new pyridoxal-5′-phosphate biosynthetic pathway**. *J Am Chem Soc* 2005, **127**:3682-3683.
Reconstitution of PLP biosynthesis by the DXP-independent pathway.

25. Hanes JW, Burns KE, Hilmey DG, Chatterjee A, Dorrestein PC,
•• Begley TP: **Mechanistic studies on pyridoxal phosphate synthase: the reaction pathway leading to a chromophoric intermediate.** *J Am Chem Soc* 2008, in press.
Mechanistic characterization of the early steps involved in PLP biosynthesis.

26. Hanes JW, Keresztes I, Begley TP: **Trapping of a chromophoric
• intermediate in the Pdx1-catalyzed biosynthesis of pyridoxal 5′-phosphate.** *Angew Chem* 2008, in press.
A novel trapping strategy of an advanced intermediate involved in PLP biosynthesis.

27. Sakuraba H, Tsuge H, Yoneda K, Katunuma N, Ohshima T: **Crystal
• structure of the NAD biosynthetic enzyme quinolinate synthase**. *J Biol Chem* 2005, **280**:26645-26648.
First reported structure of the NadA apoenzyme.

28. Cicchillo RM, Tu L, Stromberg JA, Hoffart LM, Krebs C, Booker SJ:
• ***Escherichia coli* quinolinate synthetase does indeed harbor a [4Fe-4S] cluster**. *J Am Chem Soc* 2005, **127**:7310-7311.
A combination of UV-visible, Mossbauer, and ESR spectroscopies demonstrated for the first time that anaerobically purified NadA from *E. coli* contained an Fe/S cluster.

29. Zhang Y, Colabroy KL, Begley TP, Ealick SE: **Structural studies
•• on 3-hydroxyanthranilate-3,4-dioxygenase: the catalytic mechanism of a complex oxidation involved in NAD biosynthesis**. *Biochemistry* 2005, **44**:7632-7643.
Crystal structure of 3-hydroxyanthranilate-3,4-dioxygenase in complex with 3-hydroxyanthranilate and nitric oxide clarifies the mechanism.

30. Colabroy KL, Begley TP: **The pyridine ring of NAD is formed by a
• nonenzymatic pericyclic reaction**. *J Am Chem Soc* 2005, **127**:840-841.
A model study on the ring opening of *N*,*N*-dimethylcarbamoylpyridinium with hydroxide and methoxide suggests that the pyridine ring of NAD is formed by a nonenzymatic pericyclic reaction.

31. Layer G, Moser J, Heinz DW, Jahn D, Schubert W-D: **Crystal
• structure of coproporphyrinogen III oxidase reveals cofactor geometry of Radical SAM enzymes**. *EMBO J* 2003, **22**:6214-6224.
The first crystal structure of a radical SAM enzyme.

32. Layer G, Pierik AJ, Trost M, Rigby SE, Leech HK, Grage K,
•• Breckau D, Astner I, Jaensch L, Heathcote P *et al.*: **The substrate radical of *Escherichia coli* oxygen-independent coproporphyrinogen III oxidase HemN**. *J Biol Chem* 2006, **281**:15727-15734.
EPR spectroscopic evidence for a relatively stable substrate-derived radical in the coproporphyrinogen III oxidase-catalyzed reaction.

33. Taga ME, Larsen NA, Howard-Jones AR, Walsh CT, Walker GC:
BluB cannibalizes flavin to form the lower ligand of vitamin B12. *Nature* 2007, **446**:449-453.
Structural studies on BluB clarify the mechanism of DMB formation from reduced FMN.

34. Ealick Steven E, Begley Tadhg P: **Biochemistry: molecular cannibalism**. *Nature* 2007, **446**:387-388.