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Biosynthesis of Thiamin Pyrophosphate

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Abstract

The biosynthesis of thiamin pyrophosphate (TPP) in prokaryotes, as represented by the *Escherichia coli* and the *Bacillus subtilis* pathways, is summarized in this review (Fig. 1). The thiazole heterocycle is formed by the convergence of three separate pathways. First, the condensation of glyceraldehyde 3-phosphate and pyruvate, catalyzed by 1-deoxy-D-xylulose 5-phosphate synthase (Dxs), gives 1-deoxy-D-xylulose 5-phosphate (DXP). Next, the sulfur carrier protein ThiS-COO- is converted to its carboxyterminal thiocarboxylate in reactions catalyzed by ThiF, ThiI, and NifS (ThiF and IscS in *B. subtilis*). Finally, tyrosine (glycine in *B. subtilis*) is converted to dehydroglycine by ThiH (ThiO in *B. subtilis*). Thiazole synthase (ThiG) catalyzes the complex condensation of ThiS-COSH, dehydroglycine, and DXP to give a thiazole tautomer, which is then aromatized to carboxythiazole phosphate by TenI (*B. subtilis*). Hydroxymethyl pyrimidine phosphate (HMP-P) is formed by a complicated rearrangement reaction of 5-aminoimidazole ribotide (AIR) catalyzed by ThiC. ThiD then generates hydroxymethyl pyrimidine pyrophosphate. The coupling of the two heterocycles and decarboxylation, catalyzed by thiamin phosphate synthase (ThiE), gives thiamin phosphate. A final phosphorylation, catalyzed by ThiL, completes the biosynthesis of TPP, the biologically active form of the cofactor. This review reviews the current status of mechanistic and structural studies on the enzymes involved in this pathway. The availability of multiple orthologs of the thiamin biosynthetic enzymes has also greatly facilitated structural studies, and most of the thiamin biosynthetic and salvage enzymes have now been structurally characterized.

ENZYMES INVOLVED IN THIAZOLE BIOSYNTHESIS

DXP Synthase

DXP synthase (Dxs) catalyzes the formation of DXP from glyceraldehyde 3-phosphate and pyruvate (Fig. 2A). Remarkably, this thiamin biosynthetic enzyme requires TPP as a cofactor. The crystal structure of Dxs has been solved (1) (Fig. 2B and C).

Sulfide Carrier Protein

ThiS-COSH is the sulfide donor for the thiazole biosynthesis. The enzymes involved in its formation are shown in Fig. 3, and the properties of each protein are summarized below.

The structure of ThiS-COOH has been determined (2, 3, 4). ThiS-COOH is structurally and functionally similar to ubiquitin (Fig. 4), suggesting that ubiquitin may have evolved from a

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prokaryotic sulfide transfer protein (4, 5, 6). Analogous sulfide carrier proteins have now been identified in the molybdopterin (6, 7), cysteine (8), and thioquinolobactin (9) biosynthesis pathways.

ThiS Thiocarboxylate Synthase

ThiS thiocarboxylate synthase (ThiF) catalyzes the formation of ThiS-COSH (2) as outlined in Fig. 3. The adenylation of ThiS-COOH, followed by the addition of a protein-bound persulfide, gives a mixed acyl disulfide which then undergoes reduction to generate ThiS thiocarboxylate. In *B. subtilis*, any of the IscS proteins can function as sulfur donors (10, 11), while in *E. coli*, an additional adaptor protein (ThiI) is required (12). The enzymology of the reduction of the mixed acyl disulfide has not yet been elucidated.

The crystal structures of ThiF (13) and the ThiF-ThiS complex (2) have been solved, and a model for the active site in the ThiF-ThiS complex with bound ATP has been proposed (Fig. 5) (2).

NifS (IscS) is a pyridoxal 5′-phosphate-dependent (PLP) enzyme, also involved in iron-sulfur cluster biosynthesis. The mechanism of NifS persulfide formation has been determined (14), and the crystal structure of NifS has been solved (Fig. 6) (15).

ThiI also functions as the sulfide donor for 4-thiouridine formation in tRNA (16, 12). The structure of ThiI in a complex with AMP has been solved (17, 18) (Fig. 7).

Dehydroglycine Formation

In *B. subtilis*, dehydroglycine is formed from glycine by a flavoenzyme (ThiO)-catalyzed reaction using molecular oxygen. This enzyme has been structurally and mechanistically characterized (Fig. 8) (19, 20).

E. coli can biosynthesize thiamin in the absence of oxygen. Under these conditions, dehydroglycine is formed from tyrosine in a remarkable reaction catalyzed by ThiH, recently discovered to be a radical *S*-adenosylmethionine (SAM) enzyme. This reaction has been reconstituted in a defined biochemical system (21, 22, 23).

Thiazole Formation

Thiazole synthase (ThiG) catalyzes the complex condensation of dehydroglycine, DXP, and ThiS-COSH to form the thiazole tautomer. The mechanism of the reaction catalyzed by the *B. subtilis* enzyme has been intensively studied, and the current mechanistic proposal is outlined in Fig. 9 (10, 11, 24). The aromatization of the thiazole tautomer is catalyzed by TenI (unpublished results). *E. coli* and many other bacteria do not contain a TenI ortholog. In these systems, it is likely that the aromatization occurs after the coupling of the thiazole tautomer with HMP-PP.

The crystal structure of the ThiS-ThiG complex has been determined, and an active-site model including bound DXP has been proposed (Fig. 10) (3).

The structure of the thiazole tautomerase (TenI) has also been determined (Fig. 11) (25).

ENZYMES INVOLVED IN PYRIMIDINE BIOSYNTHESIS

HMP-P Synthase

The biosynthesis of 4-amino-5-hydroxymethyl-2-me-thylpyrimidine phosphate (HMP-P) from AIR is one of the most complicated rearrangement reactions found in living systems. This reaction has been reconstituted only recently in a defined biochemical system. The pyrimidine synthase (ThiC) is a radical SAM enzyme (26), but the mechanism of the complex rearrangement is not yet known. The results of extensive labeling studies revealing the origins of the HMP-P atoms are shown in Fig. 12 (26, 27, 28, 29, 30, 31).

The structure of the *Caulobacter crescentus* pyrimidine synthase in a complex with desamino-AIR has been determined, and a reasonable model for the enzyme–desamino-AIR–SAM–Fe_4S_4 complex has been proposed (Fig. 13) (unpublished data).

HMP-P Kinase

HMP-P kinase (ThiD) catalyzes the phosphorylation of HMP and HMP-P (Fig. 14). This is a very unusual substrate tolerance for a kinase, with only one other example reported (32). The structure of HMP-P kinase has been determined and provides an explanation for this remarkable substrate tolerance (Fig. 14) (33).

ENZYMES INVOLVED IN TPP BIOSYNTHESIS

Thiamin Phosphate Synthase

Thiamin phosphate synthase (ThiE) catalyzes the coupling of carboxythiazole phosphate and hydroxymethyl pyrimidine pyrophosphate to give thiamin monophosphate (Fig. 15). The mechanism of this reaction has been characterized in considerable detail, and the intrinsic rate constant for the formation of the pyrimidine carbocation has been determined (34). A remarkable structure of thiamin phosphate synthase, with the pyrimidine carbocation trapped at the active site, has been described (Fig. 15) (35, 36).

Thiamin Phosphate Kinase

The final step in thiamin biosynthesis is the phosphorylation of thiamin phosphate, catalyzed by thiamin phosphate kinase (ThiL). The crystal structure of ThiL has been solved (Fig. 16) (37).

THIAMIN TRANSPORT

The thiamin-regulated operon *tbpA-thiBP* in *E. coli* and *Salmonella* serovar Typhimurium (*thiXYZ* in *B. subtilis*) encodes an ABC transporter involved in thiamin uptake (38). Remarkably, this transport system is capable of transporting thiamin, thiamin phosphate (TMP), and TPP (38). This substrate tolerance is highly unusual, as phosphorylated metabolites are generally not taken up by bacteria. The structure of the periplasmic thiamin binding protein (TbpA) has been determined and explains the remarkable tolerance of the thiamin transport system (Fig. 17) (39).

B. subtilis uses an additional ABC transporter corresponding to the *ykoCDEF* operon, which codes for two transmembrane components (the products of *ykoC* and *ykoE*), an ATPase (the product of *ykoD*), and a thiamin-HMP binding protein (the product of *ykoF*) (40).

THIAMIN SALVAGE

All of the stable dephosphorylated biosynthetic intermediates shown in Fig. 18 can be incorporated into TPP, and all of the indicated salvage kinases have been characterized (33, 41, 42, 43).

Recently, a new salvage pathway for HMP was identified. In this pathway, thiazole-degraded thiamin is converted to HMP, as shown in Fig. 19 (44).

The TenA protein has been structurally characterized and uses a catalytic strategy similar to that used by thiaminase I (Fig. 20). TenA is widely distributed in all three kingdoms of life but is not found in *E. coli* or *Salmonella* serovar Typhimurium (25, 44).

REGULATION OF THIAMIN BIOSYNTHESIS

The *E. coli* operons *thiCEFSGH*, *thiMD*, and *tbpA-thiBP* are regulated by a THI box, a TPP binding riboswitch (45, 46, 47, 48, 49, 50, 51, 52).

The THI box consists of a 5′ untranslated region of the mRNA that forms a thiamin binding site. In gram-positive bacteria, the binding of thiamin induces the formation of a Rho-independent transcriptional terminator. In gram-negative bacteria, the binding of thiamin masks the Shine-Dalgarno sequence which is required for the initiation of translation (53). The thiamin binding domain of this riboswitch is 1,000-fold more specific for TPP than for TMP, with K_d (dissociation constant) values of 0.1 and 100 μM, respectively (51), thereby ensuring that only the active form of the cofactor inhibits translation.

The crystal structure of a riboswitch in a complex with TPP has been resolved (Fig. 21) (54, 55, 56).

CONCLUSIONS AND UNSOLVED PROBLEMS

Since the first review of TPP biosynthesis, published in the second edition of Escherichia coli *and* Salmonella: *Molecular and Cellular Biology* in 1996 (57), our understanding of this pathway has evolved as the research has advanced from genetic and labeling studies to the mechanistic characterization of most of the biosynthetic and salvage enzymes (58, 59), and the entire pathway has now been reconstituted in a biochemically defined system. This rapid progress was greatly facilitated by high-resolution protein mass spectrometry analyses that revealed unanticipated protein posttranslational modifications (e.g., in the case of ThiS-COSH) (10) and by the availability of multiple genome sequences, making it possible to shift from a biochemically intractable protein (e.g., ThiH) in one bacterial system to a more tractable protein (ThiO) in another (20). The availability of multiple orthologs of the thiamin biosynthetic enzymes has also greatly facilitated structural studies, and most of the thiamin biosynthetic and salvage enzymes have now been structurally characterized (60, 61).

Thiamin biosynthesis is a complex story, however, and much remains to be discovered. Some of the unsolved problems are as follows.

- Several unsolved mechanistic issues regarding the bacterial enzymes remain. Foremost among these are the mechanism of dehydroglycine formation catalyzed by ThiH and the mechanism of pyrimidine formation catalyzed by ThiC.
- The structures of additional enzyme substrate-analog complexes are needed to complete our understanding of the catalytic mechanisms involved in thiamin biosynthesis. A major challenge will be determining the complete structure of the membrane-bound thiamin ABC transporter.
- The recent discovery of a pyrimidine salvage pathway from thiazole-degraded thiamin opens up the possibility that other forms of chemically degraded thiamin are also salvaged by pathways that remain to be discovered.
- Aside from dephosphorylation reactions and the oxidation of the thiamin alcohol, nothing is currently known about thiamin catabolism (62).
- Adenosine thiamin triphosphate was recently discovered as a stress metabolite formed in response to carbon starvation in *E. coli* (63). The biosynthesis pathway for this interesting molecule and its role in *E. coli* physiology remain to be discovered.
- The biosynthesis of thiamin in eukaryotes proceeds by a different route from the bacterial pathway. While considerable progress in outlining the biosynthesis of the thiazole moiety has recently been made (64, 65, 66), the biosynthesis of the pyrimidine, from pyridoxal and histidine, remains a fascinating unsolved issue (67).
- Thiamin is an important commercial chemical (produced at a rate of 3,300 tons/year), used as a food additive and flavoring agent. Thiamin biosynthesis has not yet been successfully exploited to develop a fermentation route to thiamin (9a).
- Thiamin is essential for all forms of life. The inhibition of its biosynthesis, therefore, has potential as a strategy for the development of antibiotics, particularly against bacteria lacking a thiamin uptake system (e.g., *Mycobacterium tuberculosis*) (68).

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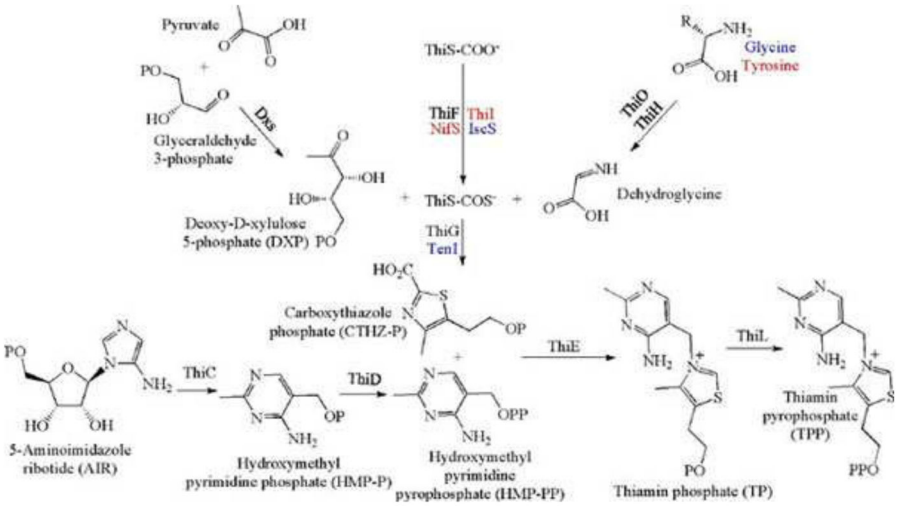

Figure 1. Prokaryotic thiamin biosynthesis

B. subtilis proteins are labeled in blue, *E. coli* proteins are labeled in red, and proteins common to both microorganisms are labeled in black. Compound abbreviations are in parentheses.

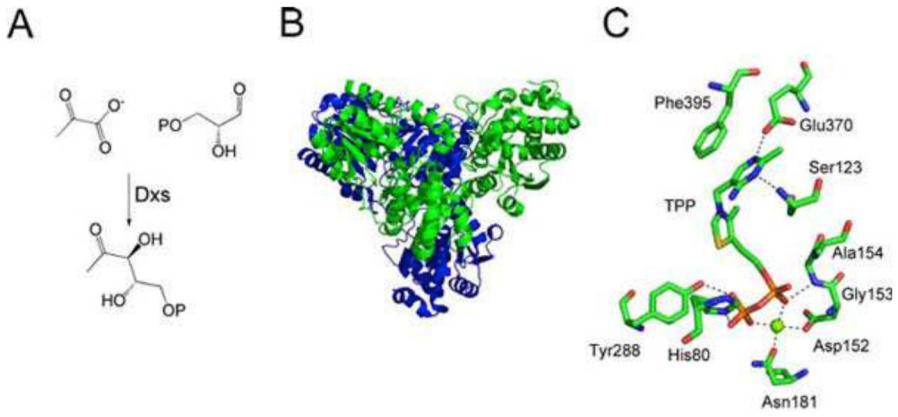

Figure 2.

(A) Reaction catalyzed by DXP synthase (Dxs). (B) X-ray crystal structure of Dxs with individual protomers labeled in blue or green (Protein Data Bank [PDB] accession no. 2O1S). (C) Model of the active site of Dxs showing the environment around the TPP cofactor.

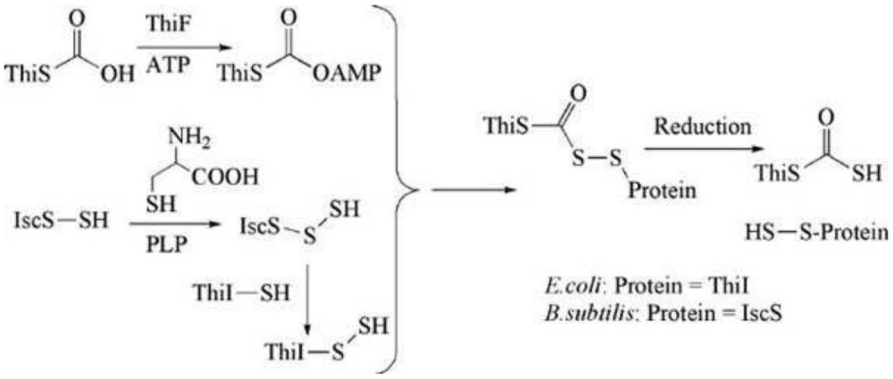

Figure 3. Reactions involved in the formation of ThiS-COSH, the sulfide source for thiazole formation

PLP, pyridoxal 5′-phosphate.

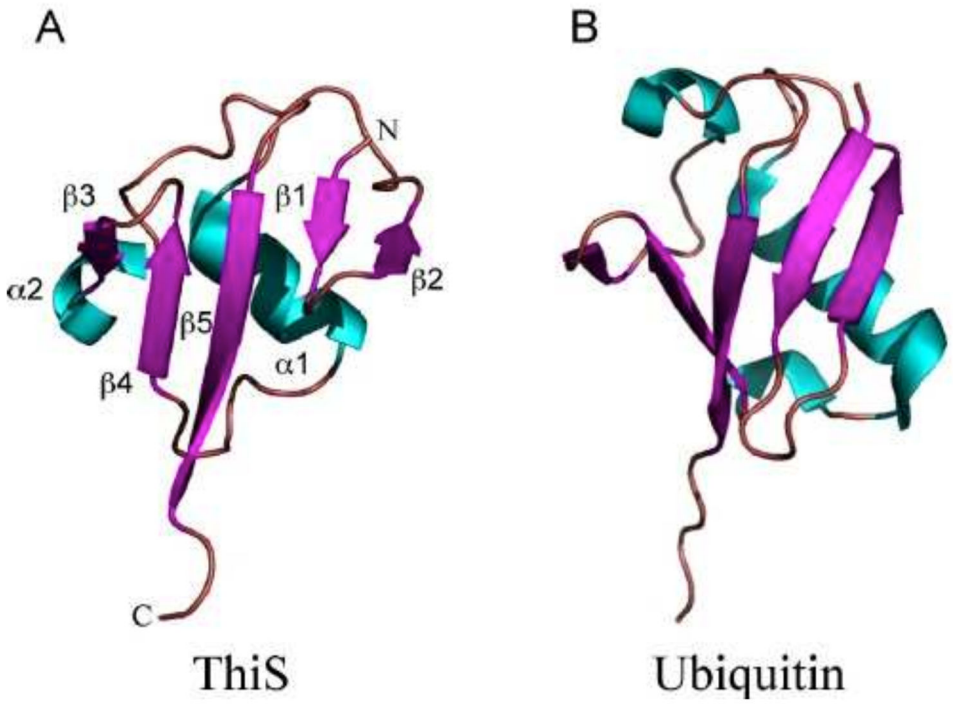

Figure 4.

(A) X-ray crystal structure of ThiS with numbered secondary structures (PDB accession no. 1ZUD). (B) NMR structure of ubiquitin (PDB accession no. 1D3Z). Helices are colored blue, and strands are colored magenta.

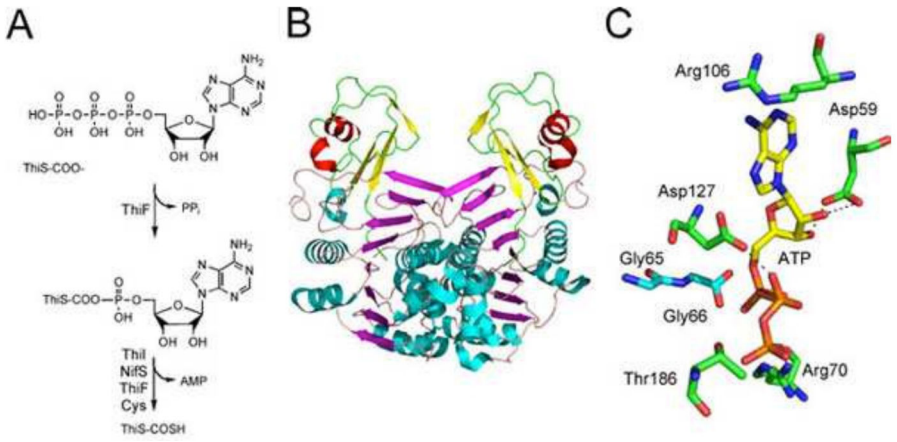

Figure 5.

(A) Reactions catalyzed by ThiS thiocarboxylate synthase (ThiF). (B) X-ray crystal structure of the ThiF-ThiS complex (PDB accession no. 1ZUD). (C) Active-site model for ThiF-ThiS showing the carboxy terminal of ThiS (Gly66) positioned close to the α phosphate of ATP.

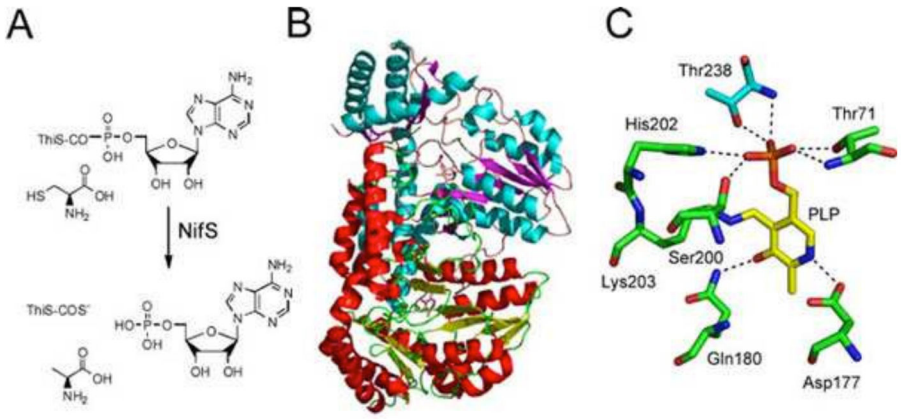

Figure 6.

(A) Reaction catalyzed by NifS. ThiS posttranslationally modified with an AMP on its C-terminus (ThiS-COAMP) and cysteine react to give thiocarboxylated ThiS (ThiS-COS$^-$) alanine and AMP. (B) X-ray crystal structure of NifS. Protomer 1 is shown with blue helices and magenta strands, and protomer 2 is shown with red helices and yellow strands (PDB accession no. 1KMJ). (C) X-ray crystal structure of the NifS active site showing the environment around the PLP cofactor. PS, perselenocysteine; SC, selenocysteine.

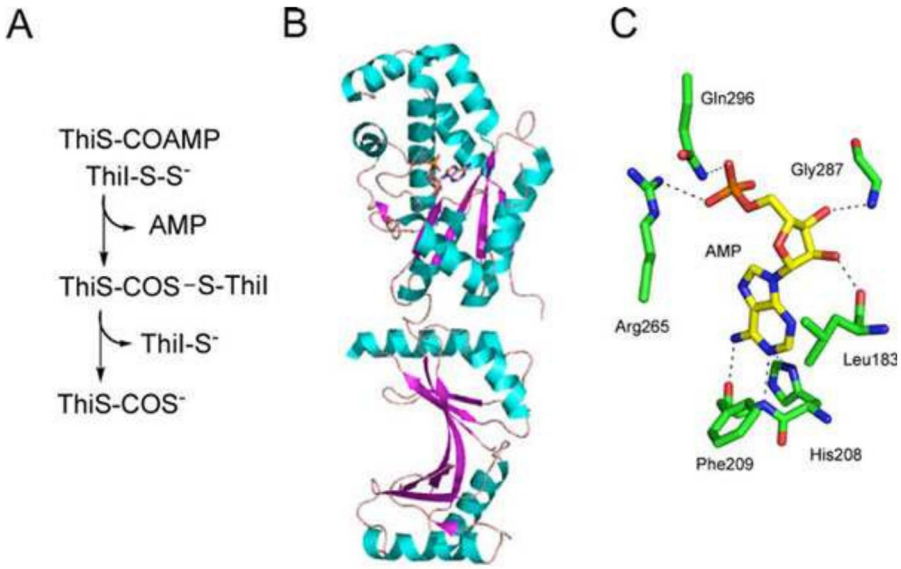

Figure 7.

(A) Reaction catalyzed by ThiI. (B) X-ray crystal structure of ThiI (PDB accession no. 2C5S). (C) Model of the active site of ThiI showing the environment around bound AMP.

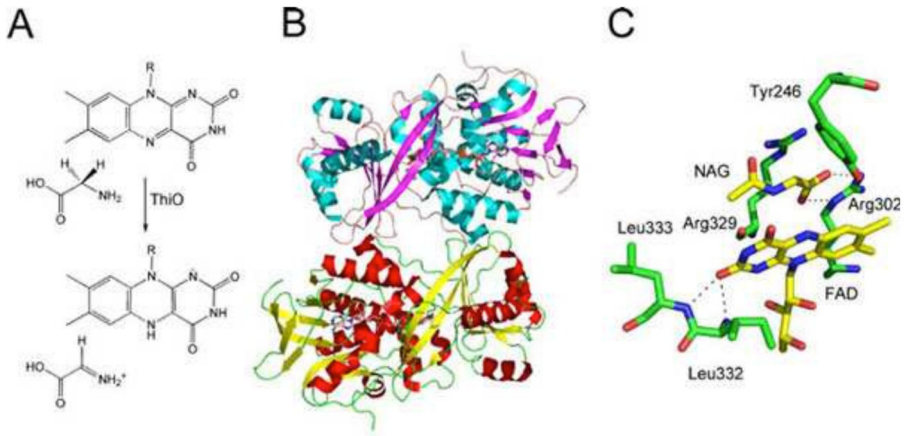

Figure 8.

(A) Reaction catalyzed by glycine oxidase (ThiO). (B) X-ray crystal structure of ThiO with flavin adenine dinucleotide (FAD) and *N*-acetyl glycine (NAG) bound in the active site (PDB accession no. 1NG3). (C) Model of the active site of ThiO showing the environment around the flavin cofactor and the stable substrate analog *N*-acetyl glycine.

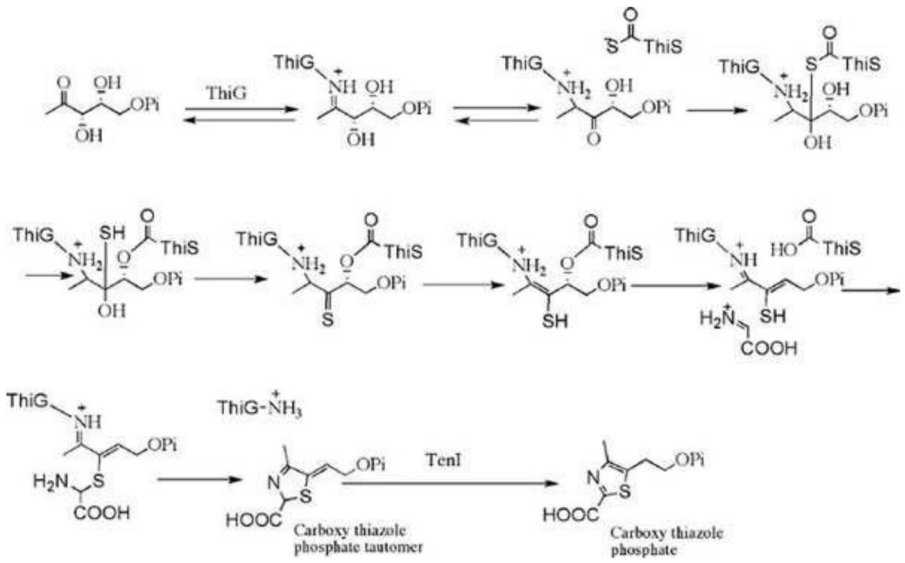

Figure 9. Current mechanistic proposal for the complex reaction catalyzed by the *B. subtilis* thiazole synthase

The last step, involving a thiazole aromatization reaction, is catalyzed by a separate thiazole tautomerase (TenI).

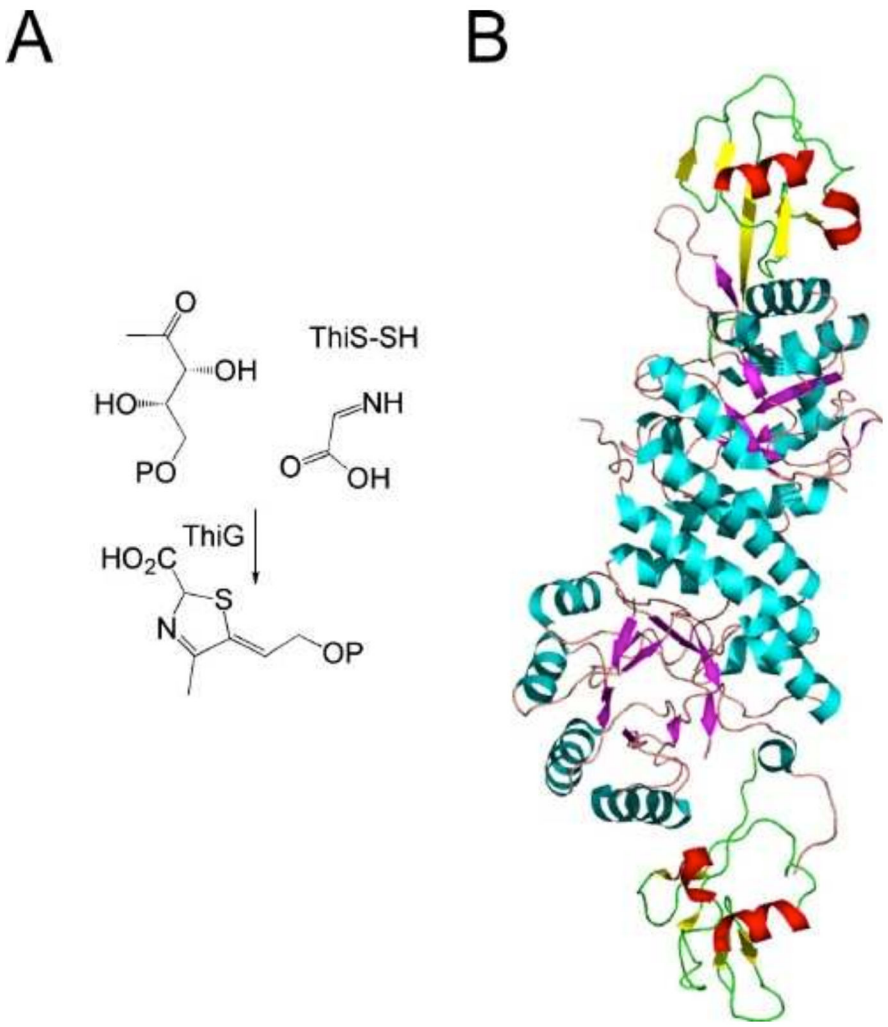

Figure 10.

(A) Thiazole synthase (ThiG)-catalyzed reaction. (B) X-ray crystal structure of the ThiG-ThiS complex. ThiG protomers are colored with blue helices and magenta strands. ThiS protomers are colored with red helices and yellow strands.

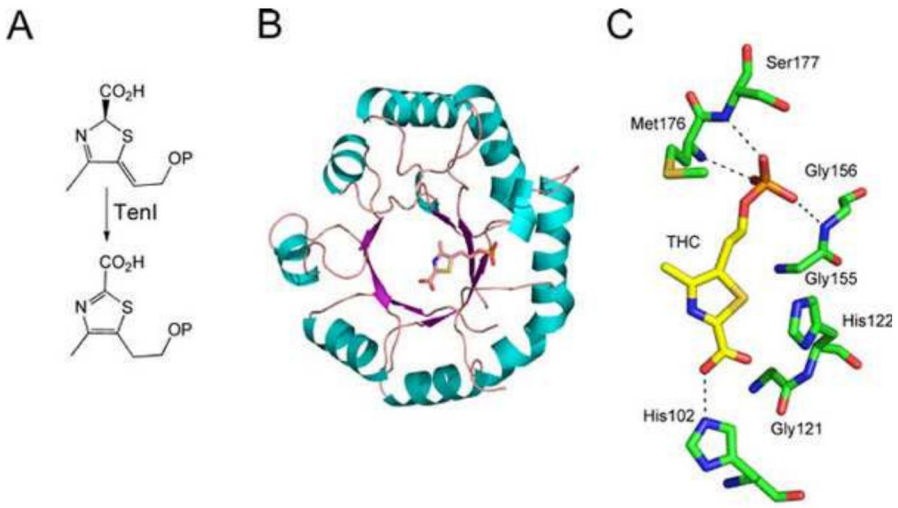

Figure 11.

(A) Reaction catalyzed by thiazole tautomerase (TenI). (B) X-ray crystal structure of TenI with thiazole carboxylate bound in the active site (PDB accession no. 1YAD). (C) Model of the active site of TenI showing the residues around the carboxythiazole phosphate reaction product. THC, thiazole carboxylate.

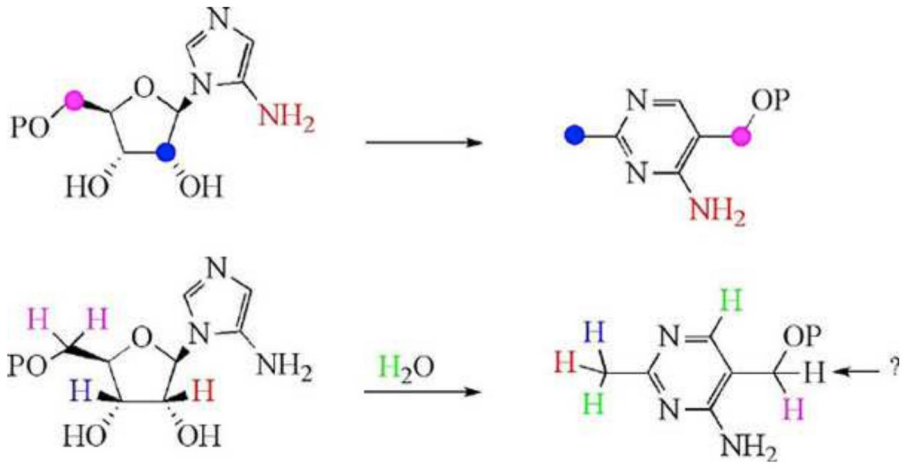

Figure 12. Studies using site-specifically labeled AIR have established the origins of all but one of the atoms of HMP-P and revealed a complex rearrangement reaction involved in the formation of HMP

The colored atoms in HMP-P are derived from the corresponding colored atoms of AIR.

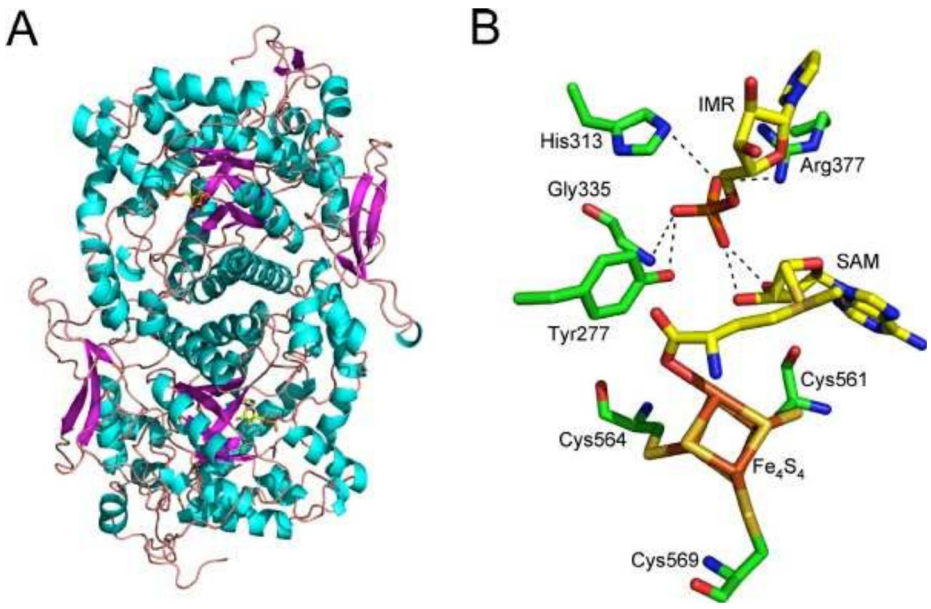

Figure 13.

(A) X-ray crystal structure of the pyrimidine synthase (ThiC) with desamino-AIR (IMR) bound in the active site. (B) Model of the active site of ThiC showing the proposed enzyme-IMR-SAM-Fe_4S_4 complex.

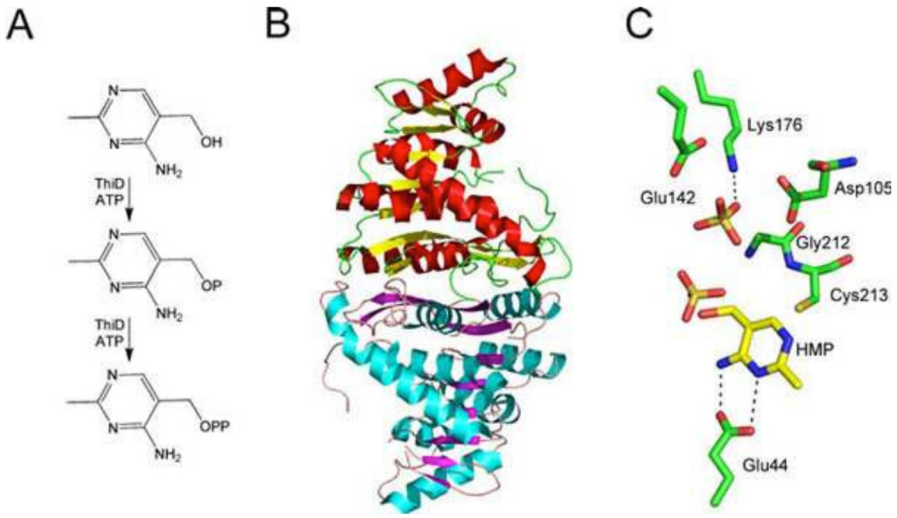

Figure 14.

(A) Reactions catalyzed by HMP-P kinase (ThiD). (B) X-ray crystal structure of ThiD (PDB accession no. 1JXI). (C) Model of the active site of ThiD showing the environment around the HMP substrate.

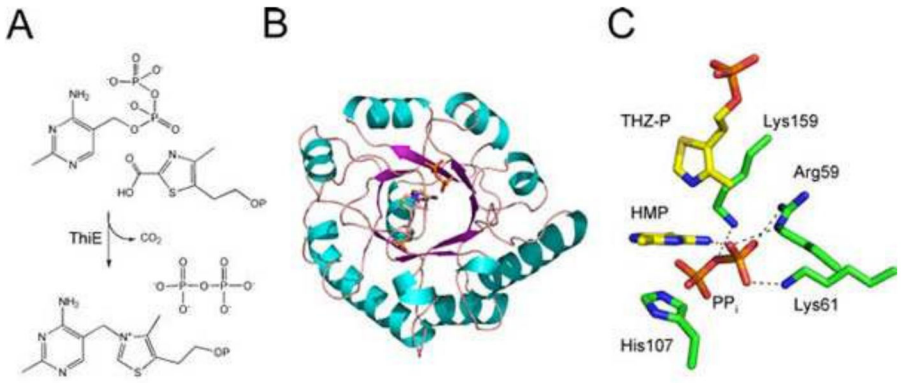

Figure 15.

(A) Reaction catalyzed by thiamin phosphate synthase (ThiE). (B) X-ray crystal structure of ThiE (S130A mutant form; PDB accession no. 1G69). (C) Model of the active-site environment showing the pyrimidine carbocation intermediate sandwiched between the pyrophosphate and the thiazole phosphate (THZ-P).

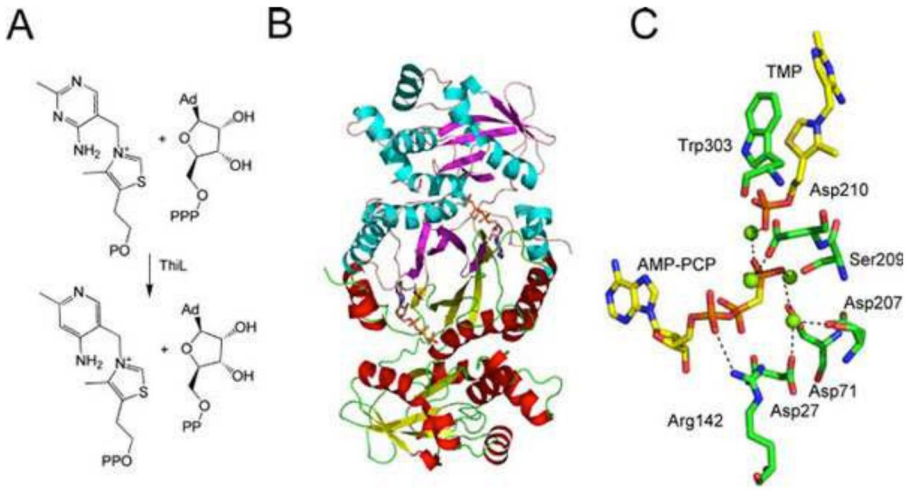

Figure 16.

(A) Reaction catalyzed by thiamin phosphate kinase (ThiL). Ad, adenine. (B) X-ray crystal structure of ThiL with the ATP analog AMP-PCP bound in the active site of each protomer (PDB accession no. 3C9T). (C) Model of the active site of ThiL showing the environment around the AMP-PCP and TMP.

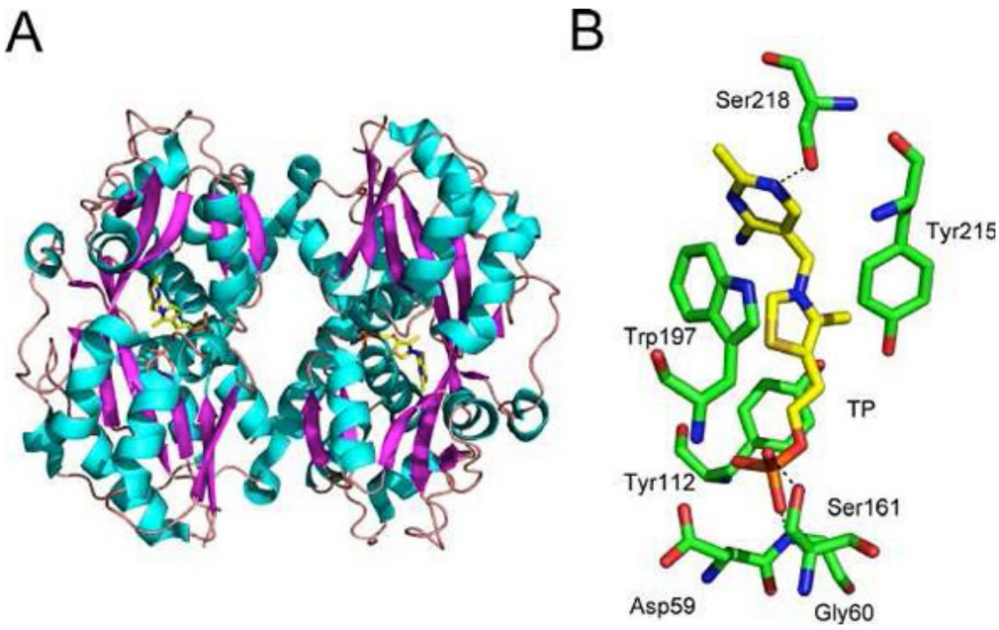

Figure 17.

(A) Structure of the*E. coli* periplasmic thiamin binding protein (TbpA) (PDB accession no. 2QRY). (B) Details of the TMP binding site.

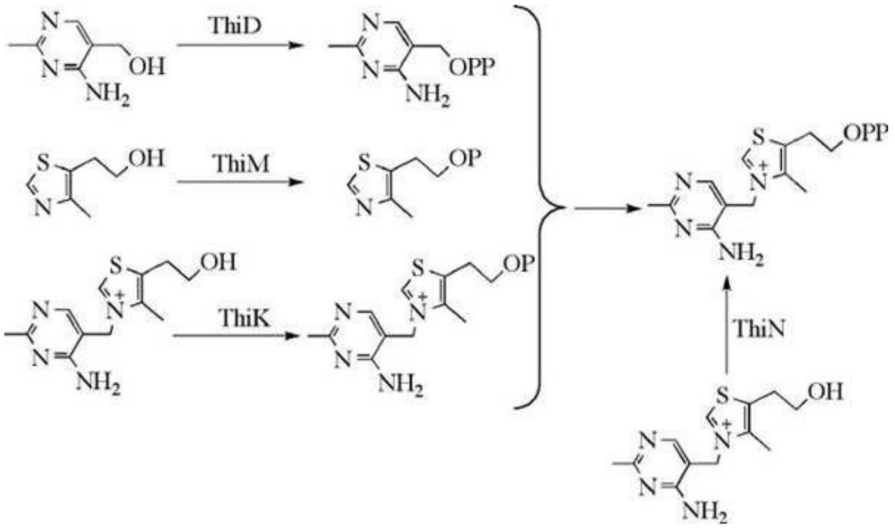

Figure 18.
Phosphorylation reactions involved in the salvage of stable dephosphorylated TPP biosynthetic intermediates.

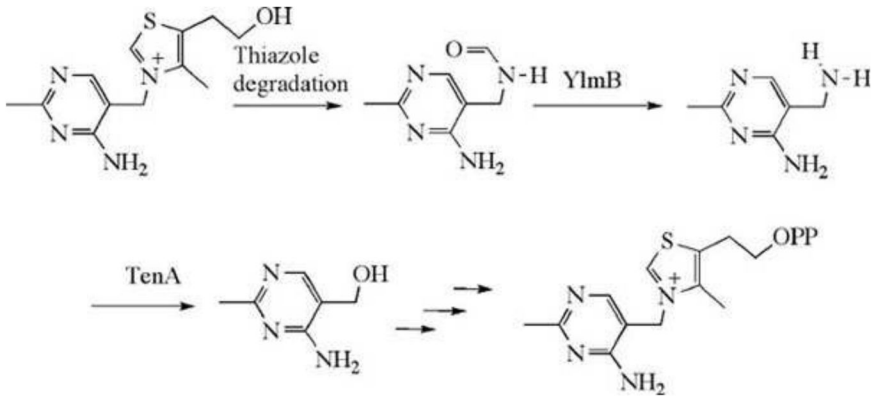

Figure 19.
New salvage pathway for HMP.

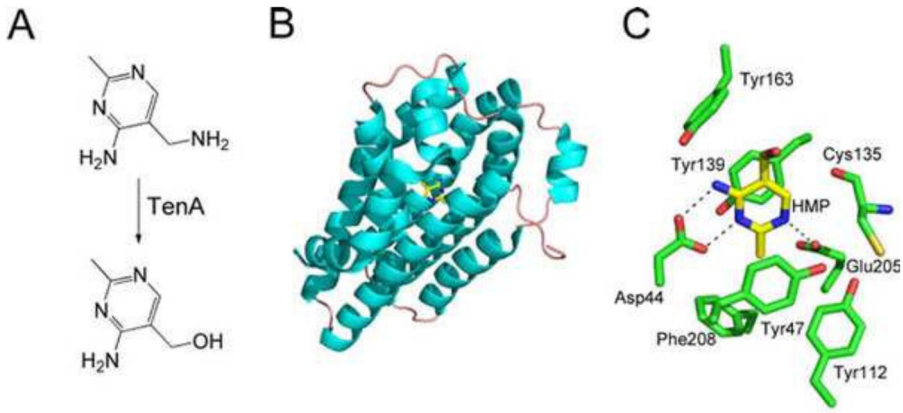

Figure 20.

(A) Reaction catalyzed by TenA. (B) X-ray crystal structure of TenA with HMP bound in the active site (PDB accession no. 1YAK). (C) Detailed view of the TenA active site in the vicinity of the bound product.

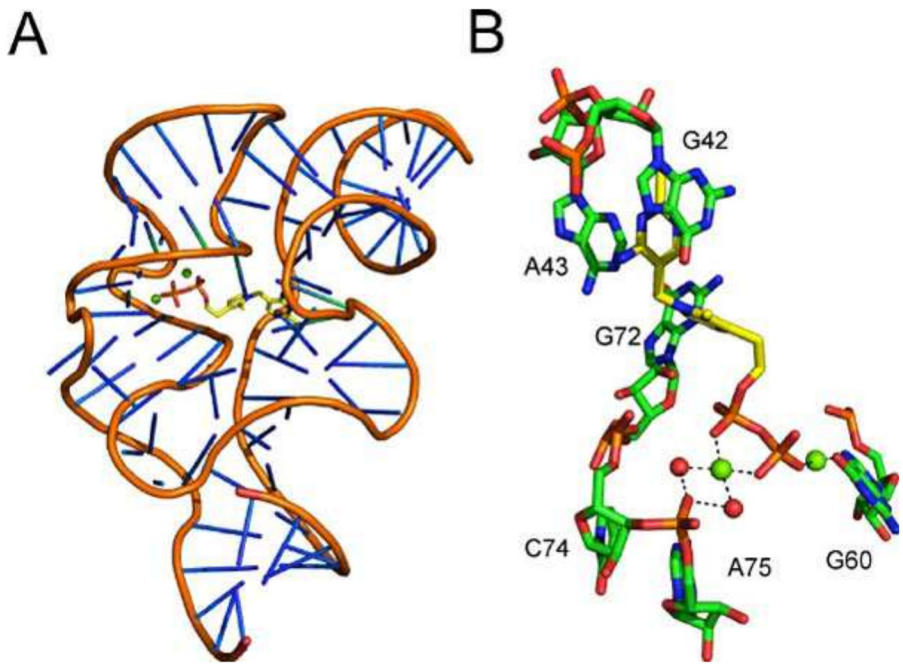

Figure 21.

(A) X-ray crystal structure of the TPP-binding riboswitch with bound TPP (PDB accession no. 2GDI). (B) Detailed view of the TPP binding site with magnesium ions depicted as green spheres and water molecules depicted as red spheres.