

Review

Thiamin (Vitamin B1) Biosynthesis and Regulation: A Rich Source of Anti-microbial Drug Targets?

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Received: 2010.10.19; Accepted: 2011.01.05; Published: 2011.01.09

Abstract

Drug resistance of pathogens has necessitated the identification of novel targets for antibiotics. Thiamin (vitamin B1) is an essential cofactor for all organisms in its active form thiamin diphosphate (ThDP). Therefore, its metabolic pathways might be one largely untapped source of antibiotic targets. This review describes bacterial thiamin biosynthetic, salvage, and transport pathways. Essential thiamin synthetic enzymes such as Dxs and ThiE are proposed as promising drug targets. The regulation mechanism of thiamin biosynthesis by ThDP riboswitch is also discussed. As drug targets of existing antimicrobial compound pyrithiamin, the ThDP riboswitch might serve as alternative targets for more antibiotics.

Key words: thiamin biosynthesis; riboswitch; *Mycobacterium tuberculosis*; drug targets

Introduction

Inexorable increase of drug resistant pathogens has exacerbated the dire outcome of recalcitrant infectious diseases due to limited effective antibiotics. A notorious case of point is the drug resistance of *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB). TB is a leading cause of death worldwide: about two million deaths and 9.4 million prevalent cases (300,000 multidrug resistant cases included) were estimated in 2008 alone [World Health Organization, 2009]. To tackle infectious diseases, especially the drug resistance infection, it is urgent to find novel antibiotics against unexplored drug targets.

To maximally potential toxic and side-effect, an ideal antimicrobial compound should target pathogen unique pathways or molecules. Biosynthetic pathways of several cofactors have been proposed accordingly as encouraging antibiotic targets such as the pantothenate kinase involving in coenzyme A biosynthesis, the lumazine synthase (LS) of riboflavin biosynthesis, and the NMN/NaMN adenylyltransferase (NMNAT), NAD synthetase (NADS), NAD ki-

nase (NADK) involving in the NAD (P) biosynthesis pathway [1-3].

Thiamin (Vitamin B₁) is indispensable for the activity of the carbohydrate and branched-chain amino acid metabolic enzymes in its active form thiamin diphosphate (ThDP). Therefore it is an essential cofactor for all organisms [4-6]. Most bacteria, as well as fungi and plants, could produce thiamin *de novo*, but mammals depend solely on the dietary uptake. Recently, the thiamin metabolism pathway of *Plasmodium falciparum*, the pathogenic agent of tropical malaria, has been suggested as a source of drug targets recently [7]. It is logical that the thiamin biosynthetic enzymes and underlying regulation network might be suitable drug targets for the development of novel antimicrobial agents.

ThDP-dependent enzymes are crucial for pathogens

ThDP-dependent enzymes such as pyruvate dehydrogenase (PDH), 2-oxoglutarate dehydrogenase

(OGDH), transketolase (TK), and acetoxyacid synthase (AHAS), are involved in various enzymatic reactions and multiple pathways. PDH bridges the glycolysis and the citric acid cycle *via* acetyl-CoA. OGDH catalyzes a rate-limiting step of the citric acid cycle. TK plays a crucial role in the pentose phosphate

pathway and AHAS participates in the branched-chain amino acid biosynthesis. These ThDP-dependent enzymes are essential for many crucial pathways and are conserved among pathogens including *M. tuberculosis* (Table 1).

Table 1. The distribution of several ThDP-dependent enzymes in *M. tuberculosis* H37Rv. Abbreviations: AHAS, acetoxyacid synthase; DXS, 1-deoxy-D-xylulose-5-phosphate synthase; InPDC, indole-3-pyruvate decarboxylase; PDC, pyruvate decarboxylase; OGDH, 2-oxoglutarate dehydrogenase; PDH, pyruvate dehydrogenase; TK, transketolase.

EC	Enzyme	ORF	Identity with human homology (amino acid)	Pathway	Known inhibitors	References
2.2.1.6	AHAS	Rv3003c Rv3509c	N.A	Branched-chain amino acid biosynthesis	Metsulfuron-methyl, sulfonylureas, imidazolines, pyrimidinythiobenzoates, phthalazin-1 (2H) -one, KHG20612	[8] [9, 10]
4.1.1.47	Dxs	Rv2682c	N.A	Thiamin, nonmevalonate and pyridoxal biosynthesis	Bacimethrin, Clomazone, 2-Methyl-3- (4-fluorophenyl) -5- (4-methoxy-phenyl) -4H- pyrazolo[1, 5-a]pyrimidin-7-one, fluoropyruvate	[11, 12] [13]
4.1.1.74 /4.1.1.1	InPDC /PDC	Rv0853c	24% (117/497)	Tryptophan catabolism /Glycolysis	N. A	N.A
1.2.4.2	OGDH	N.A*	N.A	Citric acid cycle	3-Deaza ThDP, Bacimethrin	[11, 12, 14]
1.2.4.1	PDH	Rv2497c, Rv2496c	39% (139/358)	Pyruvate metabolism	Acetylphosphinate, Thiamin thiazolone diphosphate (ThTDP), Thiamin thiothiazolone diphosphate (ThTTDP), Methylacetylphosphonate (PLThDP)	[15-17]
2.2.1.1	TK	Rv1449c	25% (162/650)	Pentose-phosphate pathway	Oxythiamin, N3'-pyridyl thiamin, bacimethrin	[11, 12, 18, 19]

N.A: Not applicable; **M. tuberculosis* is identified to be lack of OGDH[90].

However, few ThDP-dependent enzymes are microbe specific. Therefore, it is no wonder that no clinical novel antibiotics emerged from their inhibitors were reported [20]. One ThDP-dependent enzyme recently in the spotlight is AHAS. AHAS, a ThDP and FAD dependent enzyme, is involved in the synthesis of branched-chain amino acids (BCAAs) in plants, algae, fungi, bacteria, and archaea, but absent in animals. As the first common enzyme in the BCAA biosynthetic pathway, AHAS is the potential targets for herbicides, fungicides, and antimicrobial agents. In fact, many AHAS inhibitors, such as metsulfuron-methyl (Figure 3a), sulfonylureas (Figure 3a), imidazolines, pyrimidinythiobenzoates and phthalazin-1 (2H) -one, have been developed as herbicides [9]. Metsulfuron-methyl can inhibit the activity of AHAS by binding the mouth of the active site and blocking its access to the ThDP [8]. Previous studies of amino acids auxotrophic strains of mycobacteria and the AHAS mutant of *Burkholderia pseudomallei* have shown that microbial AHAS might be a drug target against infectious disease including tuberculosis [21-23]. Herbicide sulfonylureas can inhibit the *Mycobacterium avium* AHAS [24]. Several efficient inhibitors against AHAS from *M. tuberculosis* (for example, KHG20612, Figure 3a) and *Bacillus anthracis* are documented [9, 10, 25, 26].

Other ThDP-dependent enzymes shared by bacteria and animals still deserves considerable attention since there might be huge discrepancy between microbial enzymes and human counterpart in the sequence and higher structures. TK from *P. falciparum* has been reported as a novel target to treat malarial infection[27]. TK in human beings is also a promising drug target for the treatment of cancer since the suppress activity of TK against tumor cell is much more profound than that against normal cells. Many effective TK inhibitors have been identified such as the oxythiamin and N3'-pyridyl thiamin (Figure 3a) [18, 19]. 3-deazathiazin diphosphate (3-deaza ThDP) is one of the most potent irreversible inhibitors of ThDP-dependent enzymes. The only difference between this compound and ThDP is that the N-3 atom of ThDP has been replaced by a carbon atom resulting in the formation of a neutral thiophene ring in place of the thiazolium ring [14]. This neutral thiophene ring endows 3-deaza ThDP more hydrophobicity than that of ThDP thereby stronger interactions with the active site of ThDP dependent enzymes. In fact, based on the enzymatic studies of pyruvate decarboxylase from *Zymomonas mobilis*, 3-deaza ThDP binds this enzyme more rapidly and tightly compared with ThDP, ie, with a rate of approximately seven-fold faster and 2, 5000 fold more tighter [14]. Sim-

ilar results are also described in the binding with the E1 subunit of OGDH from *E. coli*, which showed a binding rate of 70-fold faster and 500-fold tighter than that of ThDP [14].

Bacterial thiamin biosynthesis

Prokaryotic thiamin biosynthesis is well-documented, which involves the formation of thiazole moiety (4-methyl-5- β -hydroxyethyl thiazole phosphate or THZ-P) and pyrimidine moiety (4-amino-5-hydroxymethyl-2-methyl-pyrimidine pyrophosphate or HMP-PP) separately [5]. Thiamin biosynthetic pathway of facultative anaerobe *E. coli* and aerobic *Bacillus subtilis* are the two best studied examples (Figure 1). In *E. coli*, THZ-P is derived from an oxidative condensation of tyrosine, cysteine, and 1-deoxy-D-xylulose 5-phosphate (DXP). Seven genes (*thiF*, *thiS*, *thiG*, *thiH*, *thiI*, *iscS*, and *Dxs*) are involved in this process [28-35]. *B. subtilis* THZ-P biosynthesis is different from *E. coli* since thiazole synthase (ThiH) is replaced by glycine oxidase (ThiO) [36], which utilizes glycine instead of tyrosine to form dehydroglycine to provide the C2-N3 unit for THZ-P.

The HMP-PP is produced from aminoimidazole ribotide (AIR) [28], an intermediate of purine biosynthesis pathway. Hydroxymethyl pyrimidine synthase (ThiC) catalyzes AIR to form hydroxymethyl pyrimidine phosphate (HMP-P), which is then phosphorylated to HMP-PP by Hydroxymethyl pyrimidine (phosphate) kinase (ThiD). THZ-P and HMP-PP are coupled to form thiamin monophosphate (ThMP) mediated by thiamin phosphate synthase (ThiE), and thiamin phosphate kinase (ThiL) catalyze a final phosphorylation step to yield ThDP, the active form of thiamin.

Thiamin salvage and transport pathways

In most microorganisms, thiamin or its components THZ-P and HMP-PP, could all be produced *via* salvage pathway (Figure 2) [38]. Thiazole alcohol (THZ) can be used to form THZ-P catalyzed by thiazole kinase (ThiM). ThiD is required for the salvage of HMP-PP from pyrimidine alcohol (HMP), while thiamin in bacteria can be converted to ThMP by thiamin kinase (ThiK) in *E. coli* or to ThDP by thiamin pyrophosphokinase (ThiN) in *B. subtilis*.

Exogenous thiamin and its components, ThiMP, ThDP, HMP, THZ, could also enter the cell *via* transporters (Figure 2) [39-41]. Some gram-negative bacteria, such as, *E. coli* and *Salmonella typhimurium*, contain an ATP-binding cassette (ABC) transporter encoded

by thiamin-regulated operon, which comprises a periplasmic thiamin-binding protein (TbpA), a transmembrane thiamin channel (ThiP), and an ATPase responsible for active transport (ThiQ) [39]. Gram-positive bacteria use another ABC transporter of operon *ykoCDEF*. This ABC transporter contains two transmembrane components YkoC and YkoE, an ATPase component YkoD, and a thiamin/HMP-binding protein YkoF [42, 43].

In addition, comparative analysis of thiamin regulatory THI elements (ThDP riboswitches) led to the identification of YuaJ, PnuT, ThiXYZ and CytX as candidate thiamin-related transporters (Figure 2) [42, 44]. It has been identified that ThiXYZ plays a role in the HMP salvage [45]. In this pathway, thiamin produced from various organisms in basic soil is firstly degraded into several components including formyl aminopyrimidine. Secondly, formyl aminopyrimidine binds to the periplasmic component ThiY, and then being transferred into the cell. Thirdly, Ylmb mediates deformylation of formyl aminopyrimidine into aminopyrimidine, and fourthly, TenA catalyzes the hydrolysis of aminopyrimidine into hydroxypyrimidine. Finally, ThiD catalyzes two phosphorylation steps to get HMP-PP.

The distribution of thiamin biosynthetic pathway in pathogenic bacteria

Landscapes of thiamin biosynthetic genes or operons of some pathogens outlined *via* comparative genomics analysis demonstrated some specific thiamin biosynthetic pathways [42]. The salvage and transport pathways are ubiquitous in most bacteria to complement thiamin *de novo* biosynthesis. This might cripple the value of thiamin biosynthetic enzymes as drug targets since this enable bacteria to obtain available exogenous thiamin. However, for those pathogens lack this salvage pathways and transporters, such as *M. tuberculosis*, promising targets can be found in the thiamin synthetic pathway [42]. Inhibitors can be designed to block the biosynthesis of thiamin, which will damage the growth and survival of bacteria. Many mechanisms might be used for inhibitors to cross the membrane of bacteria, for example, quinolones penetrate into cell through OmpF porin while chloramphenicol and fluoroquinolones can use Msp porins of *Mycobacterium smegmatis* [46, 47]. If these pathways exert more fundamental role, such as the indispensability for *Listeria monocytogenes* replication, this might enhance its drug target value [48].

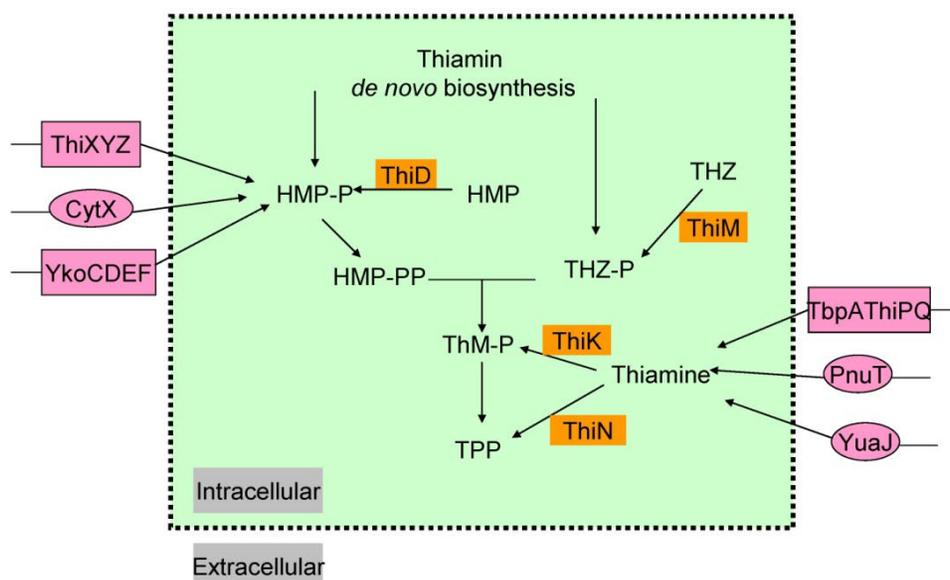


Figure 2. The salvage and transport pathways of thiamin in bacteria. Enzymes involved in the salvage of thiamin and its precursors are shown in saffron yellow background. The transport enzymes are shown in magenta background, primary and ATP-dependent transporters are in circles and rectangle, respectively. Abbreviations: ThiK, thiamin kinase; ThiM, thiazole kinase; ThiN, thiamin pyrophosphokinase.

Promising drug targets among thiamin pathway

1-deoxy-D-xylulose 5-phosphate synthase (Dxs)

Dxs catalyses the first step of thiazole biosynthesis by condensation of glyceraldehyde 3-phosphate and pyruvate to produce DXP (Figure 1a) [49]. This step also participates in the biosynthesis of pyridoxol (vitamin B₆) and isoprenoids [50, 51]. Intriguingly, ThDP is both the product of Dxp and the cofactor of Dxp. As the rate-limiting enzyme of the mevalonate-independent biosynthesis of isoprenoids, Dxs is conservative and ubiquitous among microbes, including pathogens. Therefore, it is an attractive drug target. Additionally, the resolved Dxs crystal structure from *E. coli* and *Deinococcus radiodurans* can further benefit the rational design of inhibitors against it. Dxs contains three domains (I, II, and III), each shares homology with the equivalent domains of TK and the E1 subunit of PDH [52]. Although these enzymes catalyze similar reactions and are all ThDP-dependent, the arrangement of the three domains of Dxs monomer is different from TK and PDH. The active site of Dxs is unique, which is located at the interface of domains I and II of the same monomer, instead of between two monomers as other examples.

Several inhibitors of Dxs have been identified (Figure 3b). Fluoropyruvate is described as a first inhibitor of Dxs, which is supposed to bind covalently to the active site of Dxs [13]. Dxs from *Pseudomonas*

aeruginosa and *E. coli* were inhibited by fluoropyruvate with an IC₅₀ of 400 μM and 80 μM, respectively [53]. Another known inhibitor of Dxs is 5-ketoclozazone, the metabolite of known commercially available herbicide clozazone [54, 55]. The 5-ketoclozazone was able to inhibit the activity of Dxs from *Chlamydomonas* with an IC₅₀ value of 0.1 mM [54]. However, clozazone *per se* has no effect on bacterial growth. This suggests that plants might convert clozazone into active pound 5-ketoclozazone, thereby inhibiting the activity of Dxs [56]. Based on a known transketolase inhibitor, a target-based approach has been used to identify possible *M. tuberculosis* Dxs inhibitors [57]. A preliminary structure-activity relationship (SAR) of several potential inhibitors against *M. tuberculosis* was established. So far, 2-methyl-3-(4-fluorophenyl)-5-(4-methoxyphenyl)-4H-pyrazolo[1,5-*α*]pyrimidin-7-one was the most potent Dxs inhibitor (IC₅₀ = 10.6 μM) despite its somewhat toxicity against mammalian cell [57]. Bacimethrin, an analog of HMP, is the inhibitor of a subset of ThDP-dependent enzymes including α-ketoglutarate dehydrogenase, TK and Dxs. In bacteria, bacimethrin is converted to its active metabolite 2'-methoxy-thiamin diphosphate, which would inhibit thiamin-utilizing enzymes [12]. The last three enzymes of the thiamin synthetic pathway, ThiD, ThiE, and ThiL, are all contributed to this conversion [12]. Therefore, the mutation in any of the three enzymes might alleviate the toxicity of bacimethrin to the cell. It has been tested that bacimethrin could inhibit *E. coli*

cell growth under a low micromolar range of minimum inhibitory concentration (MIC). However, this

inhibitory effect can be abrogated by low levels of exogenous thiamin or the HMP moiety [11, 12].

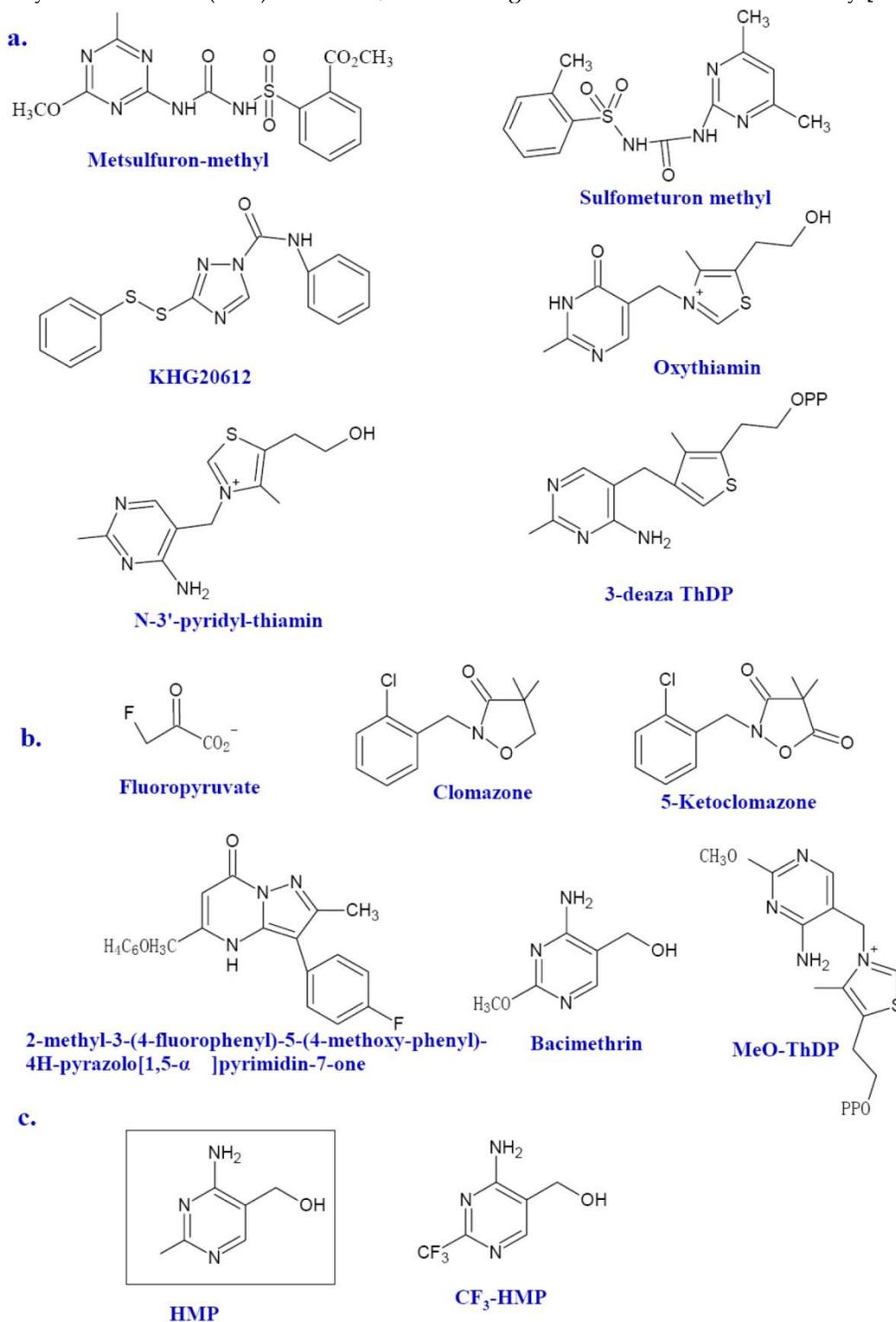


Figure 3. The structure of inhibitors. Selected inhibitors of ThDP-dependent enzymes, Dxs and ThiE are shown as a, b, and c, respectively.

Thiamin phosphate synthase (ThiE)

ThiE catalyzes the THZ-P and HMP-PP to yield ThMP. The solved ThiE crystal structures of *B. subtilis* and *M. tuberculosis* (<http://hdl.handle.net/1813/13623>) have similar structure [58]. It is dimer and has an α/β structure with a TIM barrel fold and a core of eight β -strands flanked by eight α -helices. ThiE is a crucial synthase for thiamin. The cellular trace requirement for thiamin enables it to be satisfied by enzymes with weak catalytic activity. The product of *yjbQ* from *E. coli* is a thiamin synthase homolog [59]. The *yjbQ* gene can complement the thiamin auxotrophy of *E. coli*, *Thermotoga*, *Sulfolobus* and *Pyrococcus*. However, no structural similarity exists between YjbQ and ThiE, which suggested that they might evolve from different origin. ThiE could still serve as an ideal drug target. 4-amino-2-trifluoromethyl-5-hydroxymethylpyrimidine (CF₃-HMP) (Figure 3c) is a HMP analog and could inhibit the growth of *E. coli*. In the bacteria, CF₃-HMP is converted by ThiD to CF₃-HMP pyrophosphate (CF₃-HMP-PP), an analog of HMP-PP, which inhibits the activity of ThiE [60]. Mounting thiamin level either endogenous or exogenous can reverse inhibition.

Other potential targets among thiamin biosynthetic enzymes

Among enzymes involved in thiamin biosynthesis, cysteine desulfurase (IscS) and ThiI also play a role in the 4-thiouridine biosynthetic pathways during the sulfur transfer. ThiH, ThiG, ThiF, and ThiS are genetically identified essential for *E. coli* THZ-P biosynthesis. ThiO in *B. subtilis* is essential for THZ-P formation and catalyzes the oxidation of glycine to form glycine imine [61]. ThiC is the only known enzyme to be required for the conversion of AIR to HMP-P *in vivo*, while ThiD catalyzes an essential ATP-dependent phosphorylation of HMP-P to yield HMP-PP. Other thiamin biosynthetic enzymes whose crystal structure have been determined include ThiI (PDB ID: 2C5S), IscS (PDB ID: 1P3W), ThiF (PDB ID: 1ZUD), ThiS (PDB ID: 1ZUD, 1TYG), ThiO (PDB ID: 1NG3), ThiG (PDB ID: 1TYG), ThiC (PDB ID: 3EPM), ThiD (PDB ID: 1JXI) and ThiL (PDB ID: 3C9T). These structures have shed light on their catalytic mechanisms and will contribute to the rational design of inhibitors.

Essential enzymes of the thiamin biosynthetic pathway are potential antibiotic targets. Inhibitors of these enzymes could block the endogenous thiamin biosynthesis and lead to the depletion of vitamin, thereby damaging the survival and growth of the cell. Transposon site hybridization (Trash) has identified

in vitro that thiamin biosynthetic genes *thiC*, *thiD*, *thiS*, *thiF*, *iscS*, *thiG*, *thiO* and *thiL* are essential for *M. tuberculosis* [62]. Further experiments are needed to validate their *in vivo* essentiality. Inhibitors of established essential gene products might be promising drug leads.

The regulation of thiamin metabolism by riboswitches

Riboswitches are highly conserved metabolite-sensing noncoding RNAs which are involved in the regulation of gene expression. They are mostly found in the untranslated regions of mRNAs and can bind to small-molecule metabolites directly, thereby regulating the expression of metabolic-related genes [63-66]. Riboswitches usually act in *cis* form to control the expression of their downstream genes, but two S-adenosylmethionine (SAM) riboswitches have been demonstrated to be also acted in *trans* form in addition to their *cis*-acting upon the downstream genes [67]. Typically riboswitches consist of two domains, *ie*, the aptamer domain and expression platform. The highly evolutionary conserved aptamer domain is used to recognize a target ligand while the variable expression platform can alter its structure to control genes responsive to metabolite binding [68].

Riboswitches can sense a variety of cellular metabolites whose gene products are always encoded by the riboswitch downstream sequence. These metabolites include coenzymes, amino acids, metal ions, and nucleobases [69]. ThDP riboswitch is one of the most widespread riboswitches. Members of this class have been found in bacteria, archaea, and eukaryotic organisms, regulating the expression of genes involved in the biosynthesis, salvage and transport of thiamin and its precursors [42, 70-75]. ThDP riboswitch also bears the features of other riboswitch. The resolved crystal structure of ThDP riboswitch helps the understanding of their regulation mechanisms. These structures include *thiM* riboswitch from prokaryote *E. coli* and *thiC* riboswitch from plant *Arabidopsis thaliana* [76, 77]. The high similarities of the two structures demonstrated the riboswitch to be evolutionarily conserved across diverse organisms. ThDP-driven regulation of gene expression is accomplished through interplay of two riboswitch conformations, which permit the expression of thiamin biosynthetic, transport, and salvage genes to be turned "on" or "off" (Figure 4) [78]. When the intracellular concentration of ThDP is relatively high, the aptamer domain of the riboswitch would bind to their cognate ligands and sensing domain adopts a stable metabolite-bound conformation. According to organisms, these con-

formations would act as transcription terminators or translation inhibitors, even causing mis-splicing of RNA (in eukaryotes) [79]. ThDP riboswitches are intrinsic transcription terminators and translation inhibitors. The intrinsic transcription terminator is a stable stem-loop structure followed by several consecutive uridines [80, 81]. When encountered this terminator, the ternary elongation complexes of RNA polymerase would be dissociated rapidly and thus lead to a halt transcription [69]. Therefore, the concentration of ThDP would determine whether to resume the tran-

scription or read through into the following thiamin synthetic genes upon the occupancy of the aptamer domain of riboswitch. Riboswitch can also harness ThDP binding to form a translation inhibitor, thereby regulating the access of mRNA sites near AUG start codons to the Shine-Dalgarno (SD) sequence. As a result, if the intracellular concentration of ThDP is below the threshold, ThDP would dissociate from their riboswitch, leading to the expression of ThDP synthetic genes.

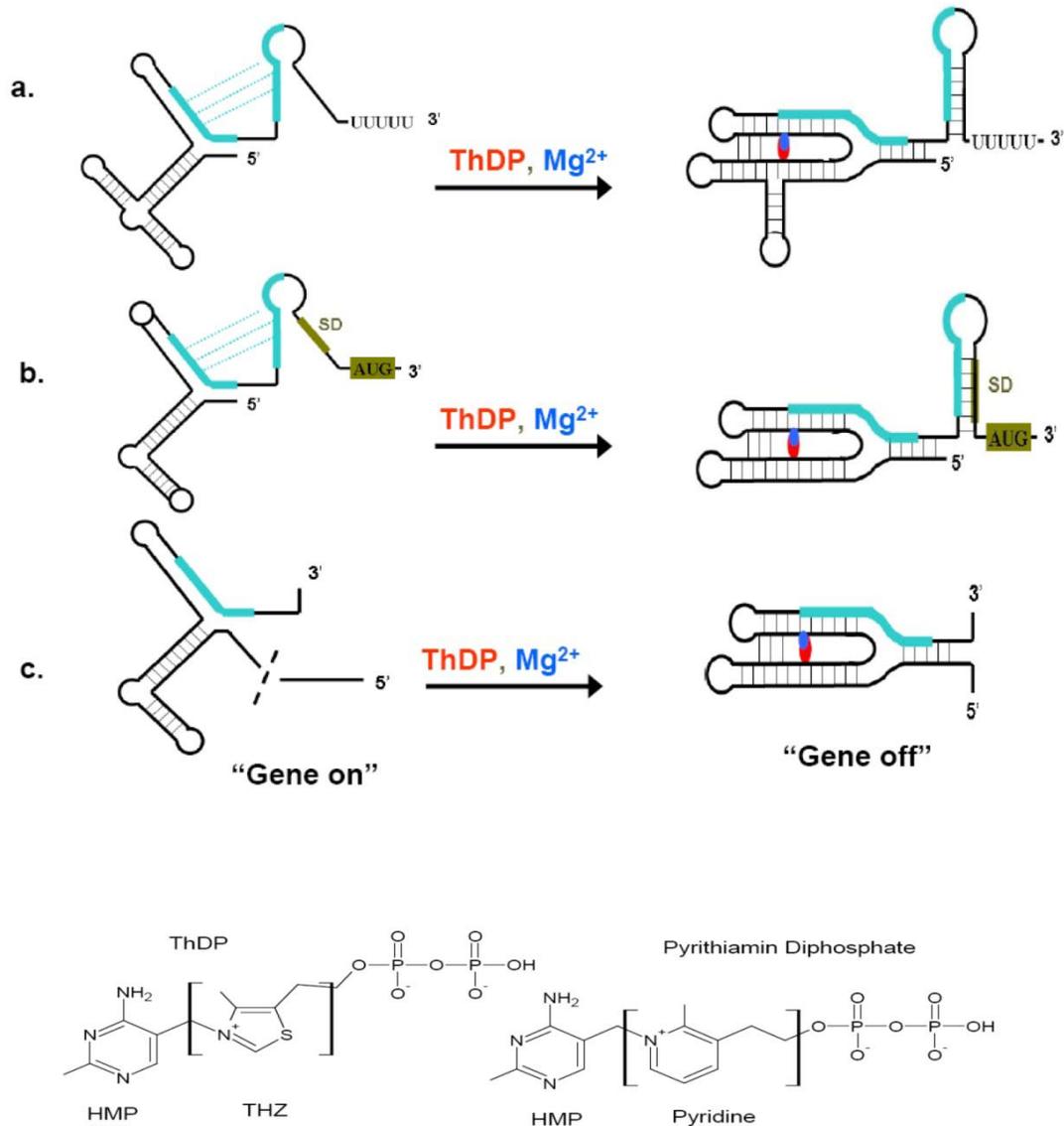


Figure 4. Regulatory mechanism of ThDP riboswitches and the structure of ThDP and Pyrithiamin diphosphate. (Top) When ThDP is at lower concentration during the synthesis of the 5'-UTR, the ThDP riboswitch would not inhibit the expression of ThDP biosynthetic genes (a, b, c, left), then "gene on". When ThDP is at higher concentration, depending on the expression platform, the ThDP riboswitch would fold to a transcription terminator (a), a suppressor of translation initiation (b), or a modulator of splicing (c), it is the "gene off" state (a, b, c, right). (Bottom) The structure of ThDP and pyrithiamin diphosphate. The initiation codon and Shine-Dalgarno (SD) sequence are shaded green brown, complementary sequences and their alternate base pairing are shown in green. The red and blue represent ThDP and Mg^{2+} ions, respectively. This figure is modified from [76, 79].

Riboswitches: ideal drug targets

It has been demonstrated that RNA molecules can fold into intricate three-dimensional structures, which will form pockets for the binding of small compounds. In this sense, the ribosome, tRNA, T box and viral RNAs have been extensively explored as antibacterial or antiviral targets [82]. However, these antimicrobial drugs merely depend on a fortuitous interaction between the compound and its cognate RNA target [83]. In contrast to above RNA, riboswitch represents a novel kind of drug targets due to their high affinity to responsive ligand.

ThDP riboswitch regulate many thiamin metabolism genes in numerous bacteria, rendering them promising drug targets for broad-spectrum antibiotics. Thiamin analog pyrithiamin exemplifies how ThDP riboswitch can act as drug target. The growth of many bacteria and fungi was inhibited by pyrithiamin. Pyrithiamin could be taken up into cell *via* thiamin transporter and further be phosphorylated into pyrithiamin diphosphate by the enzyme thiamin pyrophosphokinase. Pyrithiamin diphosphate binds ThDP riboswitch at nearly the identical affinity of ThDP, which lead to the expression of riboswitch-regulated thiamin biosynthesis, salvage or transport genes be repressed. Because the limited endogenous thiamin would soon be depleted, the survival or growth of the cell would be prevented in a short time [84]. The sole difference between pyrithiamin diphosphate and ThDP is the central thiazole ring, in which the ThDP thiazole ring was supplanted by a pyridinium ring (Figure 4). Pyrithiamin-resistant mutants of *B. subtilis*, *E. coli*, *Aspergillus oryzae* and *Chalmydomonas reinhardtii* bearing mutation in the conserved domain of ThDP riboswitch, which unambiguously demonstrated that riboswitch is the drug target for pyrithiamin [70, 74, 84]. Therefore, the wide distribution of ThDP riboswitch in several pathogens including the recalcitrant *M. tuberculosis* and promiscuous recognition of the central thiazole ring of ThDP by ThDP riboswitches render it possible to find or rationally design ThDP analogs bearing functional groups other than those linking HMP and diphosphate moieties like pyrithiamin [78].

Screening of novel riboswitch ligand analogs as selective inhibitors of some metabolic pathways also contribute to the exploring of riboswitch as drug targets. Theoretically, any inhibitor of a riboswitch could influence gene expression, regardless of the presence of its ligand [85]. Thus, according to riboswitch crystal structures, novel compounds could be designed as a source of antibiotics. Indeed, the modification of purine derivatives have resulted in the screening of sev-

eral molecules that inhibit bacterial growth[86]. It has also been identified that a pyrimidine compound (PC1) binding guanine riboswitches could kill a subgroup of bacteria [85]. More surprisingly, the fact that PC1 also showed some activity against the infection of *Staphylococcus aureus* in mouse model suggests it deserves further research to act as an antibiotic candidate. These findings have established the possibility of employing the riboswitch structures to screen novel compounds as candidates for the development of anti-microbial agents.

Discussion and Conclusion

In the search for new drug targets to combat infection, the most desirable strategy is to find compounds targeting pathogen-specific key molecules. The thiamin biosynthetic pathway perfectly matches this criterion. The Dxs and ThiE are proposed as promising enzymatic targets for novel antimicrobial agents. Other enzymes of this pathway could also serve as candidate drug targets.

Riboswitch have been extensively studied as drug targets recently [78, 87-89]. More than 20 riboswitch classes have been identified that respond to a spectrum of metabolites. Each riboswitch aptamer is featured by unique conserved primary and secondary structures [69]. ThDP riboswitch is the target of antimicrobial compound pyrithiamin. Other ThDP analogs like pyrithiamin could also bind to ThDP riboswitch and repress the expression of bacterial thiamin biosynthetic enzymes. The resolved crystal structures of riboswitch could help the designing of their inhibitors. Targeting riboswitch to develop antimicrobial agents has significant advantages. Despite distantly evolved, ubiquitous riboswitches bear conserved primary and secondary structures, which renders them ideal targets for broad-spectrum antibiotics. Moreover, multi-pronged targeting strategy against multiple riboswitches (e.g. there are *thiC* and *thiO* riboswitches in *M. tuberculosis*) can dramatically minimize the possibility of drug resistance.

The challenge is that the trace thiamin requirement of microbes can be readily compensated exogenously or by the activation of some other thiamin synthetic enzymes not yet known (for example, *yjbQ*). Therefore, the inhibition can be easily invalidated. However, for pathogens such as *M. tuberculosis de novo* synthesis of thiamin is indispensable due to absence of thiamin salvage or transporters, inhibition can be more effective. Further validation of the essentiality of genes involved in the thiamin metabolism and regulatory may reveal more potential drug targets.

Acknowledgements

This work was supported by the National key infectious disease project (Grant Nos. 2008ZX10003-006 and 2008ZX10003-001), Excellent PhD thesis fellowship of southwest university (Grant Nos. kb2009010, ky2009009 and kb2010017), the Fundamental Research Funds for the Central Universities (Grant No. XDJK2009A003), National Natural Science Foundation (grant No. 81071316) Natural Science Foundation Project of CQ CSTC (Grant No. CSTC, 2010BB5002).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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