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Chemistry and Biology of Coenzyme F_{420} in Tuberculosis treatment

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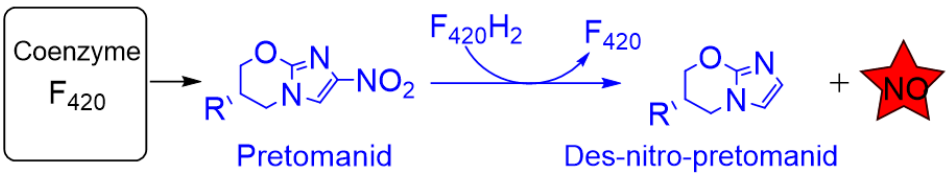
Review

ABSTRACT



Coenzyme F_{420} is one of the ancient and rare coenzymes in biology. The unique electrochemical properties of F_{420} are compared with the ubiquitous flavin coenzymes FMN (flavin mononucleotide), FAD (flavin adenine dinucleotide), and nicotinamide coenzyme NADP+ (nicotinamide adenine dinucleotide phosphate). These coenzymes participate in catalysis of different biochemical reaction involving electron/ion transfer transition in cellular metabolic pathways, which gives a significance to development of therapeutics by understanding their mechanistic participation in cellular processes. The role of F_{420} in the reductive activation of pretomanid and delamanid is used in the treatment of tuberculosis. The F_{420} is also involved in cell wall biosynthesis and reductive activation of the heme degradation pathway in *M. tuberculosis*, which contributes to the development of new drugs for the treatment of tuberculosis.

Keywords: Coenzyme F_{420}, Drug resistance, Pretomanid, Delamanid, Tuberculosis, Mycobaterium

INTRODUCTION

The coenzyme F_{420} is one of the important members of the ancient coenzymes and cofactors that participate in methanogenesis, sulfate-reduction and methanotrophic reactions in archaea and selected bacteria.[1,2] The coenzyme F_{420} is used in the biosynthesis of natural products, xenobiotic biodegradation and climate change.[1,2] The F_{420} structurally resemble the universal flavin cofactors FMN and FAD, but the specialized redox cofactor F_{420} is chemically more similar to the nicotinamide cofactors NAD and NADP. Various F420 dependent enzymes protect *M. tuberculosis* from oxidative stress reactive nitrogen species, chemistry and biology of F_{420} is important for the treatment of tuberculosis.

Tuberculosis (TB), is a communicable disease caused by slow-growing, acid-fast bacillus *Mycobacterium tuberculosis* (*M. tuberculosis*). *M. tuberculosis* is inhaled in the form of a small aerosol droplet containing the bacilli, and is transmitted to healthy individuals from an infected person through the respiratory route into the lungs. In 90% of the infected individuals, the infection does not lead to active tuberculosis. This kind of infection is called a latent tuberculosis infection (LTBI) where bacteria can live for many years in a nonreplicating state. In the remaining 10% of individuals, who are in an immune-compromised state, the disease may take the replicating form of active tuberculosis (ATB). Latent tuberculosis infection (LTBI) has become a major source of active tuberculosis (ATB).[3,4] Diagnosis of LTBI is based on the detection of an immune response to *M. tuberculosis* antigens using either the tuberculin skin test or interferon-ϒ release assays. These methods can only differentiate infected individuals from healthy ones but cannot discriminate between latent tuberculosis infection (LTBI) and active tuberculosis (ATB).[5] The application of Machine learning (ML) in the early diagnosis and prevention of LTBI and ATB represents a promising approach to accurately discriminate and diagnose LTBI and ATB.[5] There are several shared commonalities between COVID-19 and *M. tuberculosis*, particularly the transmission of their causative agents.[6–8] Both pathogens are transmitted via respiratory tract secretions. TB and COVID-19 are diseases that can be transmitted through droplets and airborne particles, and their primary target is typically the lungs.[9] Newer nano-diagnostic approaches manipulating quantum dots, magnetic nanoparticles, and biosensors for accurate diagnosis of latent, active, and drug-resistant TB are

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needed for the treatment and elimination of tuberculosis.[10–13] Streptomycin (SM) (1943) and para-aminosalicyclic acid (PAS) (1946) were among the first clinically important drugs that had been developed. The first-line anti-TB drugs are isoniazid (INH) (1952), pyrazinamide (PZA) (1952), ethambutol (EMB) (1961), and rifampicin (RIF) (1966) were introduced for clinical use. Ethambutol is included as part of the first-line RIPE (rifampicin, isoniazid, pyrazinamide, ethambutol) regimen for drug-sensitive TB. Rifampicin is a hydrophobic, high molecular-weight compound for which the mycobacterial envelope serves as a permeability barrier. By disrupting the formation of arabinogalactan, ethambutol promotes a more efficient uptake of rifampicin to exert its bactericidal effect.[14,15] Currently, the increased number of multidrug-resistant (MDR-TB), extensively-drug-resistant (XDR-TB), and in some cases drug-resistant (TDR-TB) cases raises concerns about the treatment of tuberculosis. The management of multidrug-resistant (MDR)-TB patients has been considered to be complicated and challenging because of the prolonged duration of 24 to 27 months of treatment and the high toxicity profile of second-line anti-TB drugs.[16] Fluoroquinolones are important second-line anti-Tb drugs and are active against both growing and non-growing tubercle bacilli. The molecular mechanism of action of fluoroquinolones is by inhibition of topoisomerase II (DNA gyrase), thus inhibiting subsequent DNA transcriptions and bacterial replications in MTB.[17] Second-line injectable drugs are aminoglycosides (STR, KAN, and AMK) and cyclic peptides (CAP) that inhibit protein synthesis by binding to the mycobacterial ribosome. ATP synthase inhibitor Bedaquiline was approved by the U.S. Food and Drug Administration (FDA) in 2012 for the treatment of multi-drug resistant tuberculosis.[17] A combination regimen of Pretomanid, bedaquiline, and linezolid is used for the treatment of adults with extensively drug-resistant (XDR), treatment-intolerant or nonresponsive multidrug-resistant (MDR) tuberculosis.[17] This is the first entirely new regimen for TB therapy to appear in over a half-century for controlling the global burden of the disease. Linezolid is an oxazolidinone used to treat multidrug-resistant tuberculosis (MDR-TB), including in the recently-endorsed shorter 6-month treatment regimens.[18] Molecular mechanisms may help in developing novel strategies for weakening drug resistance, thus enhancing the potency of available antibiotics against both drug-susceptible and resistant *M. tuberculosis* strains. In this brief account, the role of coenzyme F_{420} in the treatment of tuberculosis have been discussed.

COENZYME F_{420} IN TREATMENT OF TUBERCULOSIS

The mycobacterial drug discovery may be developed from two directions: target-to-drug and drug-to-target. Advances in a variety of scientific fields have enabled the amalgamation of both approaches in the development of novel drug discovery tools, which leads to accelerated drug discovery with known targets and whole cell activity.[14] Chemical-genetic studies have been a pillar of biology for decades. The simplest form of chemical-genetics relies on spontaneous mutagenesis to study the relationships between genes and drugs. Bacterial drug resistance has been classified as intrinsic drug resistance and acquired drug resistance.[14] Intrinsic resistance refers to an innate property of a bacterial species that renders an antibacterial or group of antibacterials, less effective. Intrinsic resistance mechanisms are present in almost all members of a bacterial species. In some cases, genes importing intrinsic resistance appear to have evolved specifically for protection against antibacterial compounds, whereas in other cases, genes essential for microbial growth and virulence contribute to intrinsic drug resistance. Acquired drug resistance refers to antibiotic resistance that evolves through specific chromosomal mutations or horizontal gene transfer.[14] Selected genetic techniques such as transposon mutagenesis, proteomic detection using MS analysis, and CRISPR interference chemical-genetics platforms have been developed and used to study the intrinsic drug resistance in *Mycobacterium tuberculosis*.[19] The importance of the whole-genome sequence (WGS) of M. tuberculosis strain H37Rv was reported in 1998, which improved our understanding of the genetics, pathogenicity, physiology, and metabolism of *M. Tuberculosis*.[20] Various proteins from the biosynthesis of biosynthesis of the cell wall and the energetic metabolism have been identified, but only a few bacterial proteins are targeted for antibacterial drug development.[21] A comprehensive understanding of the drug targets in M. tuberculosis may provide extensive insights into the development of safer and more efficient drugs for TB control. The cell wall and the energy metabolisms are the most important drug targets among others for the new drug for the treatment and removal of Tuberculosis.[22] Mycobacterium tuberculosis operates its energetic metabolism in a modular and compartmentalized mode to support distinct and key cellular functions. Mycobacterium tuberculosis relies on oxidative phosphorylation via the electron transport chain to produce energy for growth and division purposes. Mycobacterium bioenergetics has emerged as a promising space for the development of novel therapeutics. The unique combinations of respiratory inhibitors have been shown to have synergistic or synthetic lethal interactions, suggesting that combinations of bioenergetic inhibitors could drastically shorten treatment times.[23]

Biosynthesis of folates in *M. tuberculosis*

Many microbes including *M. tuberculosis*, are unable to acquire folates from the external environment and rely on de novo folate synthesis to support one-carbon metabolism. The folate biosynthetic pathway of M. tuberculosis begins with the synthesis of para-aminobenzoic acid (PABA) and 7,8-dihydropteridine pyrophosphate (DHPPP). PABA is produced from chorismate by the concerted action of aminodeoxychorismate synthase (PabAB) and aminodeoxychorismate lyase (PabC). DHPPP is produced from GTP via a multistep process. Production of dihydropteroate from PABA and DHPPP is catalyzed by dihydropteroate synthase (DHPS), The final enzyme of the de novo folate synthesis pathway is dihydrofolate synthase (DHFS), which catalyzes the ATP-dependent addition of L-glutamate to H2Pte to generate dihydrofolate. Dihydrofolate is reduced to tetrahydrofolate by dihydrofolate reductase (DHFR). Tetrahydrofolate serves as a cofactor for serine hydroxylase in the synthesis of glycine and is the essential precursor for various one-carbon-carrying folate species used in one-carbon metabolism.[24] Para-aminosalicylic

acid is a prodrug targeting dihydrofolate reductase (DHFR) through an unusual and novel mechanism of action.[25] The folate biosynthetic pathway offers many druggable targets that have been exploited in tuberculosis drug developments.[25] A series of small molecules have been identified that interrupt Mycobacterium tuberculosis folate metabolism by dual targeting of dihydrofolate reductase (DHFR), a key enzyme in the folate pathway, and its functional analogy, Rv2671.[25] Folates require polyglutamation to be efficiently retained within the cell and folate-dependent enzymes have a higher affinity for the polyglutamylated forms of this cofactor. Polyglutamylation is dependent on the enzyme folylpolyglutamate synthetase, which catalyzes the sequential addition of several glutamates to folate.[26] Folylpolyglutamate synthetase is essential for the growth and survival of important bacterial species, including *Mycobacterium tuberculosis*, and is a potential drug target.[26] Bacterial metabolism can cause intrinsic drug resistance but can also convert inactive parent drugs into bioactive derivatives, as is the case for several antimycobacterial prodrugs.[27] The intrabacterial metabolism of a Mycobacterium tuberculosis dihydrofolate reductase (DHFR) inhibitor with moderate affinity for its target boosts its on-target activity by two orders of magnitude.[28,29]

Chemical synthesis of pretomanid and delamanid:

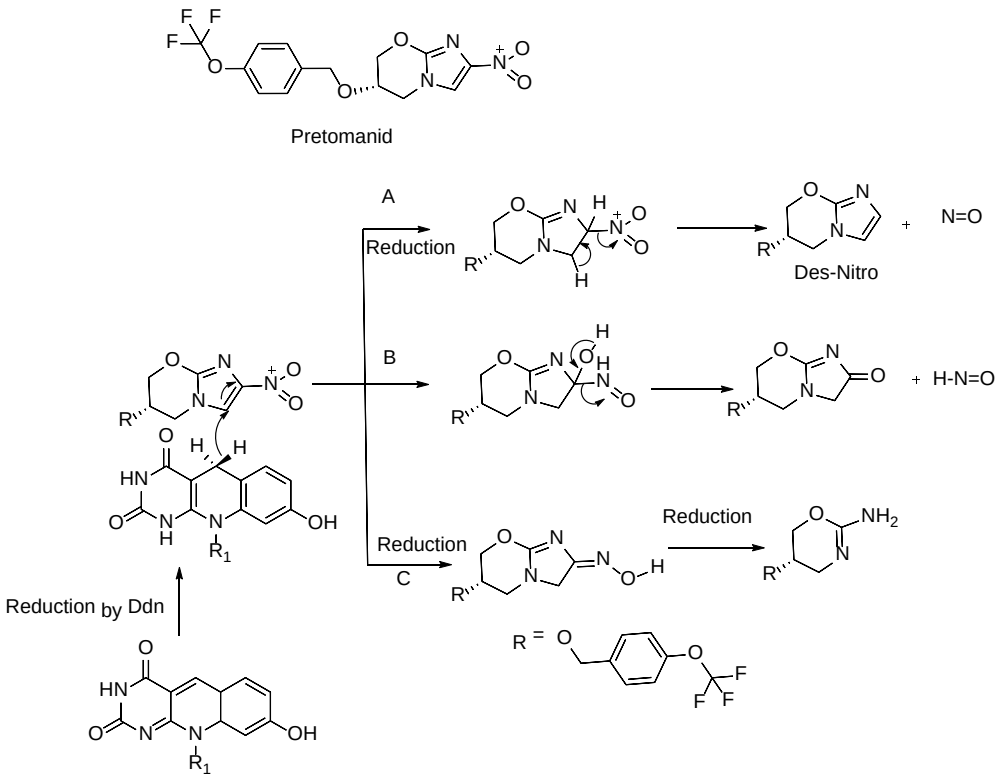
The synthesis of pretomanid (PA-824) for clinical trials was prepared in five steps, starting from the explosive 2,4-dinitroimidazole with 17% overall yield.[30] Similarly, delamanid (OPC-67683) was prepared by Otsuka Pharmaceutical Co Ltd by Tsubouchi in 16 steps.[30,31]

A solventless modification of the above pathway has also been developed.[32] A concise and convergent synthesis of pretomanid (PA-824) has been developed and this concise approach offers significant improvements over the synthetic route used for large-scale production.[33] The safe and readily available 2-bromo-4-nitroimidazole treated with a TBS-protected glycidol which undergoes a nucleophilic substitution with a TBS-protected glycidol to give protected open chain compound. This open chain compound by the installation of the aryl moiety and cleavage of the protecting group cyclized to pretomanid in 10% yield with four steps involving multiple chromatographic purifications. The novel nitroimidazopyran agent (S)-PA-824 has potent antibacterial activity against Mycobacterium tuberculosis in vitro and in vivo, where (R)-PA-824 is inactive.[34] The 2-chloro-4-nitroimidazole and (S)-epichlorohydrin open dichloro-imidazole, followed by hydrolysis to diol. Selective TBS protection of the primary alcohol enabled the benzylation of the secondary hydroxyl group to furnish alcohol and subsequent one-pot cleavage of the silyl-protecting group and cyclization afforded pretomanid in 28% over all yield.[35]

Starting from readily available protected (R)-glycidols and 2-bromo-4-nitro-1H-imidazole, pretomanid was prepared in a linear three-step synthesis in up to 40% isolated yield. In contrast to most syntheses reported so far, deprotection and cyclization were performed in a one-pot fashion without any hazardous steps or starting materials.[36] A concise, protection-group free, and sequential route has been developed for the synthesis of the delamanid starting from 2-bromo-4-nitroimidazole with an overall yield of 27%.[37]

Activation of pretomanid and delamanid

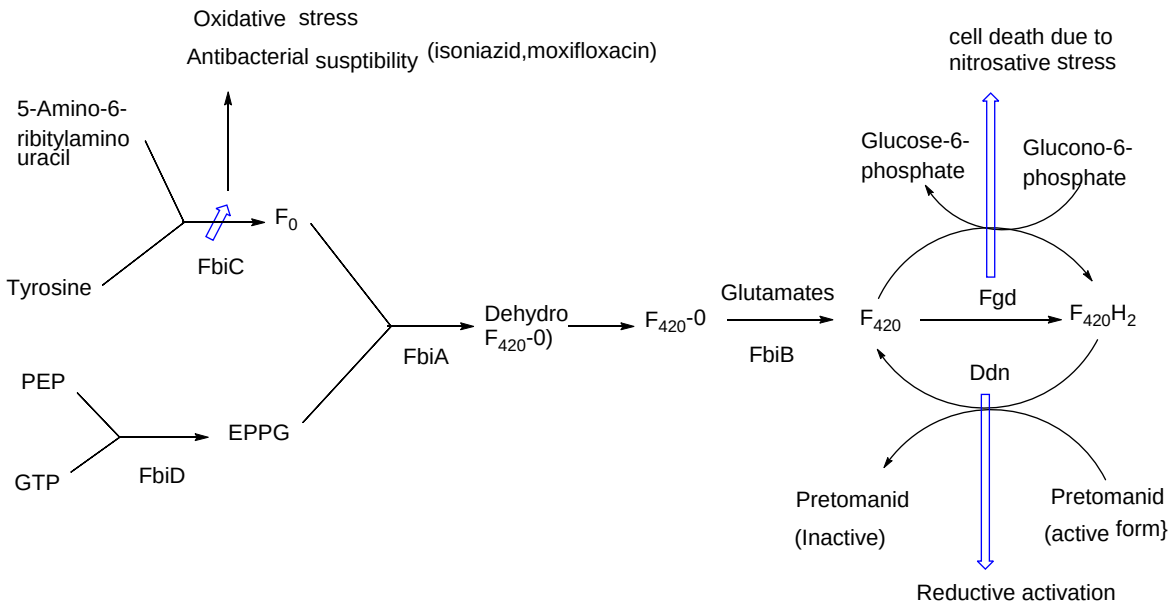
Coenzyme F_{420} is involved in glucose catabolism, respiration,[38,39] detoxifications of nitrosative stress,[40] cell wall biogenesis,[41,42] antibiotic resistance,[38,43] and cysteine biosynthesis[44] in *M. tuberculosis*. The comparative genomic methods have estimated about 28 coenzyme F_{420} dependent enzymes in *M. tuberculosis*, including 14 from the LLM family, 7 from the PPOX family, and 7 from the DDN family.[45] Bio-reductive activation of PA-824 is dependent on Rv0407, which encodes an F_{420}-dependent glucose-6-phosphate dehydrogenase.[46] In M. tuberculosis, the F_{420}-dependent glucose-6-phosphate reductase (FGD1) provides F_{420} for the in *vivo* activation of the PA-824. The structure of M. tuberculosis FRG1 has been determined by X-ray crystallography both in its apostate and in complex with F_{420} and citrate



Scheme 1: Nitroimidazole reductive activation by deazaflavin-dependent nitro reductase (Ddn)

at resolution of 1,9 and 1.95 Aº, respectively. The structure reveals a highly specific F_{420} binding mode, which is shared with several other F_{420}-dependent enzymes.[47] The resistance to PA-824 and CGI-17341 (a nitroimidazo-oxazole) is most commonly mediated by the loss of a specific glucose-6-phosphate dehydrogenase (FGD1) or its deazaflavin cofactor F_{420}, which together provide electrons for the reductive activation of this class of molecules. Although FGD1 and F_{420} are necessary for sensitivity to these compounds, they are not sufficient and require additional accessory proteins that directly interact with the nitroimidazole. The examination of the mutants that were wild-type for both FGD1 and F_{420} found that these mutants had acquired high-level resistance to PA-824, they retained sensitivity to CGI-17341. Microarray-based comparative genome sequencing of these mutants identified lesions in Rv3547, a conserved hypothetical protein with no known function. Complementation with intact Rv3547 fully restored sensitivity to nitroimidazo-oxazines and restored the ability of Mtb to metabolize PA-824. These results suggest that the sensitivity of Mtb to PA-824 and related compounds is mediated by a protein that is highly specific for subtle structural variations in these bicyclic nitroimidazoles.[48] The Rv3547 is a deazaflavin-dependent nitroreductase (Ddn) that converts PA-824 into three primary metabolites; the major one is the corresponding des-nitroimidazole (des-nitro). When derivatives of PA-824 were used, the amount of des-nitro metabolite formed was highly correlated with the anaerobic killing of *M. tuberculosis* (Mtb). Des-nitro metabolite formation generated reactive nitrogen species, including nitric oxide (NO), which are the major effectors of the anaerobic activity of these compounds. Furthermore, NO scavengers protected the bacilli from the lethal effects of the drug, Thus, these compounds may act as intracellular NO donors and could augment a killing mechanism intrinsic to the innate immune system.[49] The deazaflavin-dependent nitroreductase[50] (Ddn) from M. *tuberculosis* catalyzes the reduction of pretomanid, resulting in the intracellular release of lethal reactive nitrogen species. The N-terminal 30 residues of Ddn are functionally important but are flexible or access multiple conformations, preventing structural characterization of the full-length, enzymatically active enzyme. Mutagenesis studies based on these structures identified residues important for binding of F_{420} and PA-824.[51] The reduction of pretomanid in M. Tuberculosis is performed by enzymes belonging to the flavin/deazaflavin-dependent oxidoreductase (FDOR) superfamily,[45,49,52,53] known as the deazaflavin-nitroreductases (Ddn_s).[50,54–57] Ddn_s utilize the deazaflavin cofactor $F_{420}H_2$ as an electron source, and their deprotonated species (i.e., $F_{420}H^-$) might be the active species.[58] The cofactor $F_{420}H$ acts as a reducing agent for pretomanid in the reaction catalyzed by flavin/deazaflavin-dependent nitro reductase (Ddn).[55] $F_{420}H_2$ is generated from F_{420} in mycobacteria by the enzyme F_{420}-dependent glucose-6-phosphate dehydrogenase (FGD).[58] Thus, FGD serves to regenerate $F_{420}H_2$ for each catalytic cycle following the formation of F_{420} during pretomanid activation. The final products resulting from the reduction of pretomanid by Ddn were characterized experimentally,[53] identifying the major products as des-nitroimidazole,[59] and two other unstable products and nitrous acid, which can readily decompose and lead to nitric oxide formation which is responsible for reductive activation of pretomanid (Scheme 1).[53]

Although the metabolites isolation and characterization from delamanid has not been done, reductive activation of delamanid and the adduct formation with NAD was confirmed by comparative liquid chromatography-mass spectrometry (LC-MS) analysis of delamanid metabolites in delamanid-treated *M. tuberculosis*[53] confirmed that the activation of pretomanid and delamanid in *M. tuberculosis* follow the same pathway and both are prodrugs. The transcriptional profiling of preomanid and delamanid in M. tuberculosis demonstrates that the inhibition of cell wall synthesis and respiratory poisoning plays an important



Scheme 2: Role of antituberculosis drugs on F_{420} -dependent oxidoreductase enzymes

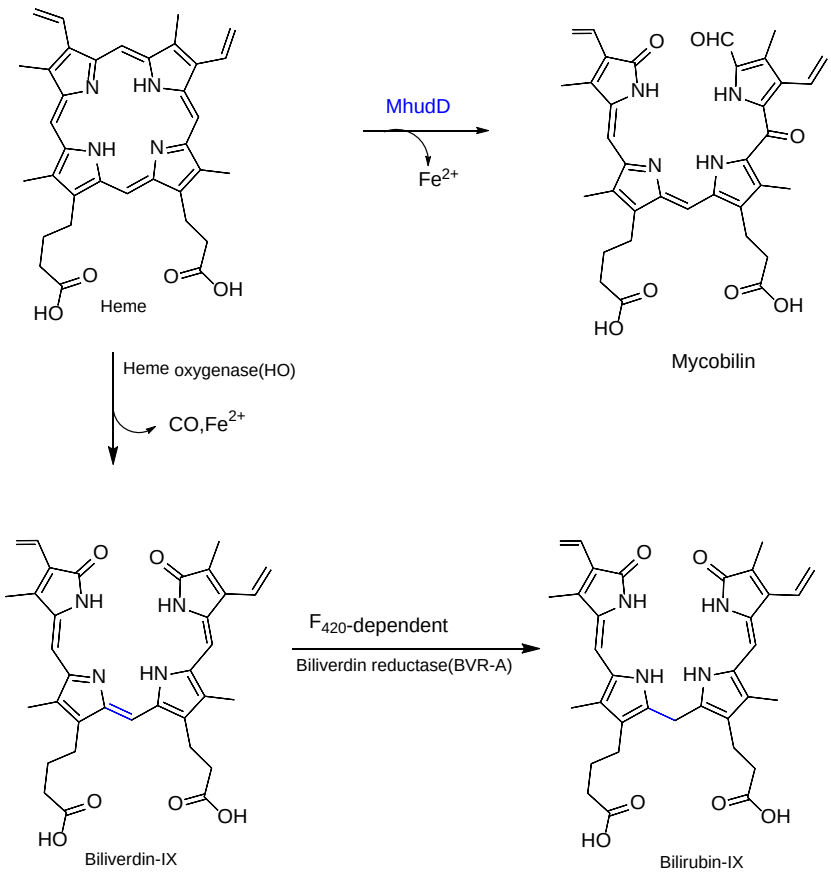
role in their bactericidal effects.[55] The activation of delamanid and pretomanid being restricted to mycobacterial Ddn might explain the selective activity against mycobacteria without being genotoxic to humans.[60]

Biosynthesis of coenzyme F_{420} in M. tuberculosis and drug resistance

Pretomanid is a narrow-spectrum nitroimidazole active against both replicating and non-replicating *M. tuberculosis.*[46] In aerobic conditions, pretomanid inhibits the biosynthesis of cell wall mycolic acid by blocking the oxidation of hydroxy-mycolate to ketomycolate, thus killing actively replicating bacteria.[46] F_{420}-dependent glucose-6-phosphate dehydrogenase is required for the activation of pretomanid.[46] Molecular analysis of the gene fgd encoded for F_{420}-dependent glucose -6-phosphate dehydrogenase(FGD) was identified in *M. tuberculosis.*[40,50,61]

Using pretomanid as a selective agent with transposon-generated in *M. bovis* strain BCG mutant with help molecular methodology demonstrated that the *M. tuberculosis* gene Rv1173(fbiC) is essential for F_{420} production and FbiC participate in the biosynthesis of F_0 and F_{420}[62–64] (Scheme 2). The genes fbiA and fbiB were also identified in coenzyme F-$_{420}$ in M Bovis BCG.[65] The Rv2983 (fbiD) a PEP guanylyltransferase FbiD) that synthesizes the phosphoenolpyruvyl-diphospho-5'-guanosine (EPPE) moiety which is subsequently transferred to Fo by FbiA to dehydro-F_{420}-0[66] (Scheme 2). FbiA encodes a transferase that is known to catalyze the transfer of a phosphoenolpyruvyl moiety to Fo to generate dehydro-F_{420}-0.[67] The FbiB encodes a bifunctional enzyme that reduces dehydro-F_{420}-0 and subsequently catalyzes the sequential addition of a variable number of glutamate residues to F_{420}-0 to yield coenzyme F_{420}-5 or -6 in mycobacteria.[67] $F_{420}H_2$ is the preferred substrate for the extension of glutamate chains of F_{420} by FbiB in *M. tuberculosis* (Scheme 2).[50] Pretomanid and delamanid are prodrugs that require metabolic activation by a deazaflavine -dependent nitroreductase (Ddn).[48] Ddn (Rv3547) converts the prodrugs into three primary metabolites, a des-nitroimidazole and two unstable by products[49] in case of pretomanid and adduct formation in the case of delamanid.[49] The Ddn is likely a membrane-bound protein[68,69] that is involved in a protective mechanism under oxidative stress. Under hypoxic non-replicating or anaerobic conditions, pretomanid is bactericidal, acting directly as a nitric oxide (NO) donor that leads to toxic NO release within the mycobacterial cells and, ultimately, respiratory poisoning.[49,53]

Both modes of action depend on nitro-reduction of the prodrug pretomanid by a deazaflavin-dependent nitroreductase (Ddn) with the reduced form of coenzyme F_{420}, which is produced by the F_{420}-dependent glucose-6-phosphate dehydrogenase



Scheme 3: Heme degradation products in *Mycobacterium tuberculosis*

fgd1[46,49,53] Thus FbiD, FbiC, FbiA, and FbiB are essential for F_{420} biosynthesis in M. tuberculosis.[50,64–66,70–72] F_{420} is synthesized by enzymes encoded by the genes fbiA, fbiB, fbiC, and fbiD.[46] Pretomanid-resistance-associated mutations have been reported in the fbiA, fbiB, fbiC, fbiD, ddn and fgd1 genes. Mycobacteria store electrons as glucose-6-phosphate(G6P) and mobilize them using Fgd (F_{420}-dependent glucose-6-phophate dehydrogenase) in response to oxidative stress[45,50,57,61,69] The $F_{420}H_2$-derived electrons may be used in endogenous redox processes to prevent damage from reactive oxygen species[45,50,57,61,69] (Scheme 2). Similarly, Delamanid-resistant bacilli have mutations in one of the 5 genes in the F_{420}-dependent bio-activation pathway with distinct F_{420} HPLC elution patterns.[73] Both genetic and phenotypic changes may be considered in the development of a rapid susceptibility test for delamanid.[73] The mutations in the genes of the F_{420} signaling pathway of the *M. tuberculosis* complex including ddn, fgd1, fbiA, fbiB, fbiC, and fbiD can lead to delamanid resistance.[74] Total RNA sequencing demonstrates that, besides the inhibition of cell wall synthesis, respiratory poisoning plays a fundamental role in the bactericidal effect of delamanid.[56] The compound identification, mechanism of action, drug resistance, in vivo activity, in vivo pharmacokinetic profiles, preclinical in vivo activity, and activity of pretomanid and delamanid are given in literature.[4661–63]

Heme degradation products :

Mycobacterium tuberculosis (MTB) infection causes acute oxidative stress and increases the expression of HO-1, which may

in turn facilitate MTB survival and growth due to increased iron availability.[75–77] MhuD is a noncanonical heme oxygenase (HO) from *M. tuberculosis* (Mtb) that catalyzes unique heme degradation chemistry distinct from canonical HOs, generating mycobilin products without releasing carbon monoxide.[75–77] Its crucial role in the Mtb heme uptake pathway has identified MhuD as an auspicious drug target. MhuD is capable of binding either one or two hemes within a single active site.[75–77] The characterization of Rv2074 from Mycobacterium tuberculosis has been reported, which belongs to a structurally and mechanistically distinct family of $F_{420}H_2$-dependent BVRs (F-BVRs) that are exclusively found in Actinobacteria.[76] Bilirubin is a potent antioxidant that is produced from the reduction of biliverdin by a novel $F_{420}H_2$ - dependent biliverdin reductase in M. tuberculosis.[76] The anti-oxidative properties of bilirubin may be involved in the pathogenesis of M. tuberculosis by conferring protection against oxidative and nitrosative species. The free iron is essential for the survival of *M. tuberculosis* (Scheme 8).[26] Drug metabolism is generally associated with liver enzymes. *M. tuberculosis*-dependent enzymes are responsible for the metabolism of drugs and play a significant role in *M. tuberculosis.*[59] Considering the range of chemical reactions involved in the biosynthetic pathways of *M. tuberculosis*, information related to the biotransformation of antitubercular compounds would provide new opportunities for the development of new chemical tools to study successful TB infections, host-directed therapy, dose optimization, and elucidation of the mechanism of action.[59]

The *M. tuberculosis* cell wall is the most complex membrane among all the bacteria. The cell wall is comprised of peptidoglycons covalently attached via a linker unit to a linear galactofuran, several strands of highly branched arabinofuran, and mycolic acids.[78,79] Mycolic acids are oriented perpendicular to the membrane and provide a lipid barrier and stability of the cell wall of *M. tuberculosis.*[78–80] *M. tuberculosis* has a complex cell wall containing mycolic acids (MA), which play an important role in pathogenesis, virulence, and survival by protecting the cell against harsh environments.

Pretomanid inhibits the biosynthesis of mycolic acids[41] (Scheme 2), whereas delamanid inhibits the synthesis of ketomycolates and methoxymycolates.[60]

The coenzyme F_{420} is one of the ancient coenzymes in biology. The coenzyme F_{420} is also involved in the microbial metabolism of 2,4,6-trinitrotoluene.[81] Tetracyclines are The hydroxylation and reduction of anhydrotetracycline are catalyzed by a FAD-dependent anhydrotetracycline hydroxylase and an F_{420}-dependent dehydrotetracycline reductase,[82,83] whereas the cofactor F_0, a synthetically accessible derivative of cofactor F_{420}, can replace F_{420} in tetracycline biosynthesis.[82,83] Aflatoxins are polyaromatic mycotoxins that contaminate a range of food crops as a result of fungal growth and contribute to serious health problems in the developing world because of their toxicity and mutagenicity. *Mycobacterium smegmatis* enzymes utilize the $F_{420}H_2$ to catalyze the reduction of the α, β-unsaturated ester moiety of aflatoxins, activating the molecules for spontaneous hydrolysis and detoxification.[84]

Conclusion

The bacterial F_{420} is biosynthesized and reduced by different enzymes in *Mycobacterium tuberculosis.* F_{420}-dependent reactions of *Mycobacterium tuberculosis* contribute to the virulence of this bacterium. The coenzyme carries a glutamic acid-derived tail, the length of which influences the metabolism of *M. tuberculosis*. The reductive activation of pretomanid by flavin/deazaflavin- dependent nitro-reductase (Ddn) formed the major products as des-nitroimidazole and nitrous acid, which can readily decompose and lead to nitric oxide formation. The anaerobic formation of nitric oxide may be responsible for antibacterial activity. Pretomanid also inhibits the formation of ketomycolicacids from hydroxymycolicacids.

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Conflict of Interest Statement

There is no conflict of interest for this review work.

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