Characterisation of $F_{420}$ gamma-glutamyl ligase from *Mycobacterium tuberculosis*

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- MOHE / MOE
TB and *Mycobacterium tuberculosis*

- *M. tuberculosis* is the major aetiological agent causing human tuberculosis (TB)
- 2011 – 8.7 million new and relapsed TB infections
- 13% involved co-infection with HIV
- Ranks second only to HIV among infectious killers worldwide
- Transmitted by air – active bacilli in air droplets expelled by people affected with pulmonary TB
- Problems in combating TB: limitations in current TB therapies, persistence, multi-drug resistance, etc..

Structural biology to understand fundamental of *M. tuberculosis*

- To determine the 3-dimensional structure of protein at molecular level (atomic resolution)
- X-ray crystallography may provide hypotheses of functional activities of the protein of interest
Our research focuses on coenzyme $F_{420}$, a flavin cofactor

- 7,8-didemethyl-8-hydroxy-5-deazaflavin
- Archaea (methanogenesis) & bacteria
- At least 28 $F_{420}^{-}$-dependent enzymes identified in *M. tuberculosis*
  
- Molecular structure very similar to FMN

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**F$_{420}$ biosynthesis**

- **FbiC (rv1173):** HPP + Compound 6 → FO intermediate (FO synthase)
- **FbiA (rv3261):** FO intermediate → F$_{420}$-0 (phospholactate transferase)
- **FbiB (rv3262):** F$_{420}$-0 → F$_{420}$-1 (glutamyl ligase)

**Key Points:**
- In mycobacterium species, the predominant structures contain F$_{420}$-5 and F$_{420}$-6 glutamate groups.
- Polyglutamylated F$_{420}$ is a conserved feature observed in reactions by F$_{420}$-dependent enzymes.
FbiB

- **BLASTp**: Comprises 2 domains
  - N-terminal domain:
    - annotated as a gamma-glutamyl ligase (Pfam PF01996)
    - 37% sequence identity with an archaeal γ-glutamyl ligase (PDB ID 2G9I)
  - C-terminal domain:
    - sequence-similarity to FMN-dependent nitroreductase family (Pfam PF0081)
    - Has been hypothesized to facilitate elongation of polyglutamate tail of $F_{420}$ in mycobacterial species (NCBI CDD TIGR03553)
- It is postulated that N-terminal domain would add up to 2 glutamate residues to the coenzyme as seen in methanogens, and the C-terminal domain would add more glutamate residues to the coenzyme

FbiB cloning, expression, purification, and crystallisation

- Gateway cloning (6XHis-tagged, GST-tagged)
- Readily expressed and purified as full length and individual domain constructs
- In both *E. coli* and *M. smegmatis* vectors
- However, only its C-terminal domain was successfully crystallized
- The 3-dimensional structure of FbiB C-terminal domain was determined to 1.75Å resolution
- Phased by multiwavelength anomalous dispersion (MAD) methods using bromide-soaked crystals
C-terminal domain X-ray data and Structure determination

Top: Apo-crystals of FbiB C-terminal domain (refined to 1.75Å)

Top: FMN-soaked FbiB C-terminal domain crystals (refined to 1.9Å)

Bottom: $F_{420}$-0 soaked FbiB C-terminal domain (refined to 1.9Å)

Diffraction image of Left: apo-FbiB C-terminal domain crystals
Right: FMN-soaked FbiB C-domain crystals
Crystal data for different crystal forms of FbiB C-terminal domain

<table>
<thead>
<tr>
<th></th>
<th>Apo-FbiB C-domain</th>
<th>FbiB C domain-F₄₂₀-0 complex</th>
<th>FbiB C-domain-FMN complex</th>
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<tbody>
<tr>
<td><strong>Space group</strong></td>
<td>P4₁2₁2</td>
<td>P4₁2₁2</td>
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<tr>
<td><strong>Unit cell dimensions</strong></td>
<td>a = b = 136.89 Å</td>
<td>a = b = 136.62 Å</td>
<td>a = b = 137.14 Å</td>
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<tr>
<td></td>
<td>c = 102.09 Å</td>
<td>c = 101.75 Å</td>
<td>c = 101.40 Å</td>
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<td>α = β = γ = 90°</td>
<td>α = β = γ = 90°</td>
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<tr>
<td><strong>No. of molecules in</strong></td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>the asymmetric unit</td>
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<tr>
<td><strong>Solvent content (%)</strong></td>
<td>54.50</td>
<td>54.23</td>
<td>54.38</td>
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<tr>
<td><strong>Vm (Å³/Da)</strong></td>
<td>2.70</td>
<td>2.69</td>
<td>2.70</td>
</tr>
</tbody>
</table>

- Contain two dimers in the asymmetric unit
Structure of FbiB C-domain dimer

- Top and side view of the FbiB C-domain dimer
  - The antiparallel beta strands at the protein core are flanked by helices.
  - Monomer A is rainbow-coloured from its N-terminus (blue) to C-terminus (red) whereas monomer B is in grey.
  - Displays a fold that is typical of FMN-dependent nitroreductases family

Refinement statistics: $R_{\text{factor}}/R_{\text{free}} (%) = 22.3/25.7$, rmsd = 0.010Å, 97.5% residues in most favoured regions, one ramachandran outlier
Electron density map around FMN and $F_{420}-0$ molecule (contoured at 1.5 sigma)
Different binding sites for FMN and $F_{420}$-0 molecule

Overlay of FMN-bound and $F_{420}$ bound structures:
Left: The protein dimer is represented as a ribbon diagram and ligands as stick models
Right: The protein dimer as a surface representation with ligands as spheres on the right
Summary

• Full length FbiB and its individual domain constructs readily expressed and purified in both *E. coli* and *M. smegmatis*

• Only C-terminal domain of FbiB has been successfully crystallised

• The structure of apo-FbiB C-terminal domain, as well as FMN-bound and F$_{420}$-0-bound FbiB C-terminal domain has been solved

• Closer inspection revealed different conserved binding sites for FMN and F$_{420}$-0 ligand

• As FMN is not a natural ligand for FbiB enzyme, its role will need to be further investigated
Thank you for your attention
TB and *M. tuberculosis*

- can remain dormant for a long time before becoming infectious when the immune system of the host is compromised
- a non-sporulating bacterium, yet can remain dormant within the human host for years
- can survive in hypoxic (oxygen-depleted) and nutrient-depleted media

Relevance of studying coenzyme $F_{420}$ in mycobacteria

- It plays important roles in particular condition such as hypoxia – may be relevant to mycobacterial persistence
- Low-cross activation by mammalian enzymes – make $F_{420}$-related proteins promising drug targets
- Structural information on enzymes from the $F_{420}$ biosynthesis pathway might provide information on how this coenzyme is regulated in mycobacteria, and how they might be developed as therapeutic targets
Solution of apo-FbiB C-terminal domain structure

• Solved using multi-wavelength anomalous dispersion (MAD) methods for phase determination

• Halide (bromide) soaked FbiB C-terminal domain crystals – display significant anomalous signal that can be used as a reference to locate the positions of these atoms