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# Snapshots of catalysis: Structure of covalently bound substrate trapped in *Mycobacterium tuberculosis* thiazole synthase (ThiG)

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ABSTRACT

Increasing drug resistance in *Mycobacterium tuberculosis* (*Mtb*) has necessitated the design of new anti-mycobacterial drugs with novel targets. Thiazole synthase (ThiG) is an essential enzyme and a potential drug target in *Mtb* that catalyzes the formation of the thiazole moiety of thiamin-pyrophosphate from 1-deoxy-D-xylulose-5-phosphate (DXP), dehydroglycine and ThiS-thiocarboxylate. To uncover the catalysis mechanism and design potent and selective anti-mycobacterial compounds targeting ThiG, we determined the crystal structure of *Mtb*ThiG at 1.5 Å resolution, for the first time, snapshotting a covalently bound substrate trapped in the catalytic pocket. The structure showed a $(\beta/\alpha)_8$ barrel overall fold as well as the dimer form of *Mtb*ThiG existing in solution. In the central pocket, Lys98 is the key residue forming a protonated carbinolamine intermediate, a functional Schiff base precursor, with DXP. The carbinolamine is further stabilized by active site residues mainly through hydrogen bonds. This work revealed that a protonated carbinolamine is initially formed and then it is dehydrated to the imine form of Schiff base during the early catalysis steps. Our research will provide useful information for understanding the ThiG function and lay the basis for future drug design by targeting this essential protein.



## 1. Introduction

According to the latest WHO's global tuberculosis (TB) report, TB is the ninth leading cause of death worldwide ranking above HIV/AIDS [1]. Increasing drug resistant TB is a continuing threat due to limited effective antibiotics [2]. In addition to developing more effective drug combination regimens to treat the multi-/extensively/totally drug-resistant TB [3], it is also necessary to develop novel drugs against new targets.

Thiamin (vitamin B1) is an essential cofactor for all microorganisms and its biosynthesis pathways have been proposed as a rich source of antimicrobial drug targets [4]. The bacterial thiamine biosynthesis is well studied [5–9], and the key enzyme thiazole synthase (ThiG), which is involved in the catalytic reaction of thiazole ring formation through intermediate DXP interacting with ThiS-thiocarboxylate, is essential for mycobacterial survival [10,11]. The catalytic mechanism of ThiG involves Schiff base formation between lysine residue in the active pocket and a substrate carbonyl [6]. The *Mycobacterium tuberculosis* ThiG (*Mtb*ThiG) was proposed as a possible drug target of pyrazinoic acid (POA) [12], the active form of pyrazinamide, and this proposition is further supported by our recent *Mtb* protein array binding study in which POA was found binding to ThiG among other proteins [13]. However, there is no information for the substrate binding and catalytic mechanism at the molecular level of this target, which is valuable for drug development by targeting ThiG.

In this study, we determined the crystal structure of *Mtb*ThiG at 1.5 Å resolution, which is covalently bound with its substrate DXP

*Abbreviations: Mtb*, *Mycobacterium tuberculosis*; ThiG, Thiazole synthase; DXP, 1-deoxy-D-xylulose-5-phosphate; POA, pyrazinoic acid; CSS, complexation significance score; PDB, Protein Data Bank.

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through forming a carbinolamine intermediate. This model revealed that ThiG transiently forms a protonated carbinolamine with DXP before dehydrating to the imine form of the Schiff base during catalysis process.

## 2. Materials and methods

### 2.1. Expression and purification of the recombinant MtbThiG

The coding sequence of ThiSG complex (Rv0416-Rv0417) from *M. tuberculosis* was amplified by PCR and cloned into pET28a vector with 6 x His tag at the N-terminus of ThiS. *E. coli* strain BL21 (DE3) (Novagen) was used as the expression host, and the overexpression and purification protocol was similar to that of the *Bacillus subtilis* ThiSG complex [9]. Bacteria were grown at 37 °C in LB broth containing 50 μg/mL kanamycin to an $OD_{600} = 0.6$ with shaking, and then induced with 0.2 mM IPTG at 16 °C for 16 h. Cells were harvested by centrifugation, resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 5% (v/v) glycerol, 1 mM PMSF) containing 20 mM imidazole, disrupted by High Pressure Homogenizer and then centrifuged for 30 min at 18,000 rpm to remove cell debris. The lysate was applied to a Ni-NTA column. After washing with lysis buffer containing 50 mM imidazole, the proteins were eluted with lysis buffer containing 250 mM imidazole. To obtain the pure ThiG eliminating ThiS, the proteins were further applied to a HiTrap Q column (5 mL, GE Healthcare). Finally, the obtained ThiG protein was loaded onto a Superdex 200 column (GE Healthcare, Life Sciences) pre-equilibrated with 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 5% (v/v) glycerol. The peak fractions were then concentrated to 6 mg/mL and stored at −80 °C.

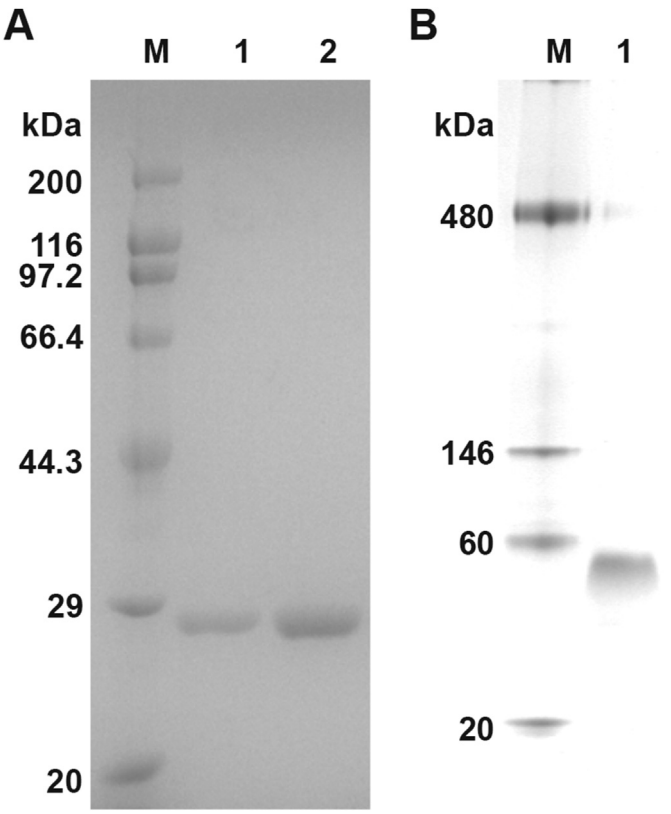


**Fig. 1.** SDS-PAGE and BN-PAGE analysis of *Mtb*ThiG.
(A) SDS-PAGE of the purified *Mtb*ThiG. Lane 1 and Lane 2 were the peak fractions from the Superdex 200 column. M: protein standard marker. (B) BN-PAGE analysis of the native polymerization state of *Mtb*ThiG.

### 2.2. SDS-PAGE and blue native PAGE (BN-PAGE) analysis

SDS-PAGE was performed to analyze the purity and molecular mass of the recombinant ThiG protein. Following the BN-PAGE protocol [14], 30 μg of ThiG protein and Native Mark™ unstained protein standard (Novex, Thermo Fisher Scientific) were loaded onto the 3–12% Bis-Tris linear gradient gel (Invitrogen) and subjected to electrophoresis according to the manufacturer's instruction. After electrophoresis, the gel was stained with colloidal Coomassie staining.

### 2.3. Crystallization

Crystallization of the purified *Mtb*ThiG was initially screened with commercially available kits including Hampton Research and Molecular Dimensions using the sitting-drop vapor-diffusion method at 16 °C. Crystals appeared from several crystallization conditions after 2–3 days. After optimization by hanging-drop vapor-diffusion method, crystals of the best quality were obtained in the condition containing 0.2 M sodium malonate pH 5.0, 20% (w/v) PEG3350 at 16 °C for 3 days.

### 2.4. Data collection and structure determination

The cryoprotectant solution was prepared by supplementation with 30% (v/v) glycerol in the reservoir solution. The crystals were fished out with a sizeable loop after transferring to cryoprotectant for a few seconds, and then flash-cooled in liquid nitrogen. Diffraction data were collected on beamline BL19U1 at Shanghai Synchrotron Radiation Facility (Shanghai, China). Data were processed using XDS [15]. The structure was determined by

**Table 1**
Data collection and refinement statistics.

| | ThiG-DXP |
|---|---|
| **Data collection** | |
| Space group | $P6_122$ |
| Wavelength (Å) | 0.97852 |
| Cell dimensions | |
| *a*, *b*, *c* (Å) | 16.67, 116.67, 123.32 |
| α, β, γ (°) | 90, 90, 120 |
| Resolution (Å)[a] | 61.66–1.48 (1.51–1.48) |
| $R_{merge}$ | 0.087 (2.349) |
| $R_{pim}$ | 0.014 (0.521) |
| Mean $I/\sigma(I)$ | 26.2 (1.4) |
| $CC_{1/2}$ | 1.000 (0.511) |
| Completeness (%) | 99.7 (95.6) |
| Redundancy | 36.1 (20.4) |
| **Refinement** | |
| Resolution (Å) | 58.34–1.48 |
| No. reflections | 82151 |
| $R_{work}/R_{free}$ (%) | 16.3/18.9 |
| No. atoms | |
| Protein | 3358 |
| DXP | 26 |
| Water | 351 |
| Average B factors (Å$^2$) | |
| Protein | 25.32 |
| DXP | 33.97 |
| Water | 38.37 |
| R.m.s. deviations | |
| Bond lengths (Å) | 0.021 |
| Bond angles (°) | 1.855 |
| Ramachandran Plot Statistics (%) | |
| Favored regions | 98.5 |
| Allowed regions | 1.5 |
| Disallowed regions | 0 |

[a] Values in parentheses are for highest-resolution shell.

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