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Crystal structure of the Ser147Gln mutant of diadenosine tetraphosphate phosphorylase from *Mycobacterium tuberculosis*

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1 Introduction

The crystal structure of diadenosine tetraphosphate (Ap_4A) phosphorylase (MtAPA), a product of *Rv2613c gene*, from *Mycobacterium tuberculosis* H37Rv revealed that Ser-147 of MtAPA is probably important for its catalytic activity [1]. To understand the role of Ser-147 in the catalysis, we constructed a Ser147Gln substituent of MtAPA (S147Q) and determined its crystal structure.

2 Experiment

S147Q was expressed and purified as described previously [1]. S147Q was crystallized by the hanging drop vapor diffusion method using a 24-well plate. Specifically, 5 μL of protein (10 mg·mL^{-1}) was mixed with 5 μL of crystallization solution (0.1 M sodium cacodylate (pH 6.3), 0.2 M lithium sulfate, and 28.5% polyethylene glycol 400), and the drop was suspended on a siliconized coverslip over 0.6 mL of the crystallization solution. Prismatic colorless crystals of S147Q formed after approximately 2 weeks at 20 °C and grew to a maximum size of 0.3 mm. Diffraction data for S147Q were collected at the AR-NW12A station. The diffraction data were processed, merged, and scaled using the HKL2000 (DENZO and SCALEPACK) software package. The crystal structure of S147Q was determined by the molecular-replacement method using MOLREP. The coordinates of the wild type MtAPA structure (PDB code 3ANO) were used as the search model. A model of S147Q was built using the Coot program, and refined using CCP4, CNSsolve, and PHENIX to a resolution of 2.79 Å. The model was validated using MolProbity.

3 Results and Discussion

The crystals of S147Q belonged to the monoclinic space group *C*2. The asymmetric unit contained 2 subunits, with a Matthew's coefficient of 2.37 Å^3 Da^{-1} and a solvent content of 48.1%. The data-collection statistics are summarized in Table 1. The crystal structure of S147Q was determined by the molecular-replacement method. The final model of S147Q consisted of 325 residues, 72 water molecules, 3 phosphate ions, 2 tetraethylene glycols, and 1 glycerol in the asymmetric unit, which included subunits A and B (S147Q-A/-B). Electron density was present for all residues in S147Q-A, except for the histidine tag (His-tag) and residues 1–13, 36–54, and 171–175. Similarly, electron density was present for all residues in S147Q-B, except for the His-tag and residues 1–23. In addition, residues 196–198 in S147Q-B were derived from the expression vector. The refinement statistics are summarized in Table 2. The structure factors and atomic coordinates for S147Q have been deposited in the PDB under the accession code 3WO5. The structure-function analysis of S147Q is now in progress.

Table 1: Summary of data collection and processing

Wavelength (Å)	1.00000
Temperature (K)	100
Space group	*C*2
Cell dimension (a, b, c) (Å)	101.0, 63.6, 79.0
(β) (deg)	111.0
Resolution range (Å)	50.0–2.79 (2.84–2.79)^a
Total No. of reflections	78447
No. of Unique reflections	11269 (518)
Redundancy	7.0 (3.4)
Completeness (%)	96.1 (87.4)
Mean $I/\sigma(I)$	21.3 (4.9)
R_{merge}^{b}	7.4 (19.0)
Wilson B-factor (Å)	24.3

^{a}Data in the highest resolution shells are listed in parentheses.
$^b R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $Ii(hkl)$ is the ith intensity measurement of reflection hkl and $\langle I(hkl)\rangle$ is its average.

Table 2: Structure-refinement statistics

Resolution range	31.8–2.79 (3.07–2.79)
Completeness (%)	95.4
Final R-factor (%)^a	17.5 (21.4)
Final R_{free} (%)^b	23.5 (27.3)
Root mean square deviation from ideality	
Bonds (Å)	0.005
Angles (deg)	0.885
Ramachandran plot	
Favored region (%)	97.2
Allowed region (%)	2.8
Outlier region (%)	0

$^a R\text{-factor} = \sum_{hkl} ||F_{obs}| - k|F_{calc}|| / \sum_{hkl} |F_{obs}|$, where k is the scaling factor.
^{b}This value is based on 5% of data excluded from refinement at random.

References

[1] S. Mori, etal., *J. Mol. Biol.* **410**, 93 (2011)

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