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Crystallographic analysis of diadenosine 5′, 5′′′-P^1, P^4-tetraphosphate phosphorylase from *Mycobacterium tuberculosis* H37Rv

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Introduction

Recently, we reported the molecular characterization of Rv2613c, a protein that is encoded by the *Rv2613c* gene of *Mycobacterium tuberculosis* H37Rv, and revealed that Rv2613c is a diadenosine 5′,5′′′-P^1,P^4-tetraphosphate (Ap_4A) phosphorylase [1]. The amino acid sequence of Rv2613c contained a histidine triad (HIT) motif consisting of H-phi-H-phi-H-phi-phi, where phi is a hydrophobic amino acid. This feature is quite unique among phosphorylases because the HIT motif has been reported to be the characteristic structure of several nucleotide hydrolases including yeast diadenosine polyphosphate hydrolase rather than phosphorylases, which usually contain H-X-H-X-Q motif as reported in yeast Ap_4A phosphorylases. Furthermore, the amino acid sequence of Rv2613c is more homologous to that of yeast diadenosine polyphosphate hydrolase than to that of yeast Ap_4A phosphorylase. These observations indicate that Rv2613c is a unique Ap_4A phosphorylase with a primary structure homologous to that of Ap4A hydrolase rather than typical Ap4A phosphorylases. In order to analyze the more detailed structure-function relationship of Rv2613c, elucidation of the crystal structure is required.

Methods

Rv2613c was cloned into pCold I vector, expressed in *Escherichia coli* BL21(DE3)pLys, and purified to homogeneity by two-step column chromatography as previously described [1]. The purity of the preparation of Rv2613c was greater than 95% as judged by the results of N-terminal amino acid sequence analysis and mass spectrometry. The purified Rv2613c was dialyzed against sample buffer (HEPES-Na, pH 7.6, and 0.5 mM dithiothreitol), and then concentrated to 10 mg ml^{-1} by an Amicon Ultra-15 at room temperature.

Sitting-drop crystallization experiments were set up in a 96-well Intelli-Plate. Crystallization condition was determined using the commercially available crystallization screening kits. The reservoirs and drop compartments of 96 well plates were filled with 60 μl and 1 μl reservoir solution, respectively, and then 1 μl purified Rv2613c was added to the drop compartment.

Diffraction data of Rv2613c were collected at the AR-NW12A station of Photon Factory using ADSC Quantum 210r detector. The crystal was harvested using a CryoLoop, flash-cooled to 100 K under a nitrogen stream, and subjected to analysis. The 360 consecutive images were collected using an oscillation range of 1° and an exposure time of 2 s at a wavelength of 1.0000 Å. The data of Rv2613c crystal were processed, merged, and scaled using program package HKL2000.

Results

The prismatic colorless Rv2613c crystals formed in about two weeks at 293 K, growing to maximum 0.5 mm under the optimum condition (0.1M sodium cacodylate, pH 6.5, 0.2 M lithium sulfate, and 30% polyethylene glycol 400). Data collection statistics for the crystal are summarized in Table 1. The Rv2613c formed a homotetramer consisting of 25 kDa subunits in solution [1]. Two subunits per asymmetric unit yields a V_M of 2.41 $Å^3$ Da^{-1} and a solvent content of 49.1%. V_M and solvent content lie within the range usually found for protein crystals. These data collection statistics indicated that this Rv2613c crystal is suitable to solve the structure. Attempts to solve the structure of Rv2613c by either multiple isomorphous replacement or MAD method using selenomethionine-labelled Rv2613c are in progress.

Table 1 X-ray diffraction data-collection statistics
(Values in parentheses are for highest resolution shell.)

X-ray source	Photon Factory
Beamline	BL-NW12A
Oscillation angle (°)	1
Exposure time (s)	2
Wavelength (Å)	1.0000
Temperature (K)	100
Space group	*C*2
Unit cell parameters	
(Å)	a = 101.5, b = 63.6, c = 79.1
(°)	β = 110.9
Resolution (Å)	50.0 – 1.90 (1.93 – 1.90)
Unique reflections	37314 (1826)
Average redundancy	7.4 (6.4)
Competeness (%)	99.8 (98.3)
Mean $I/\sigma(I)$	42.2 (4.4)
R_{merge} #	0.061 (0.380)

#$R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl and $\langle I(hkl)\rangle$ is its average.

References

[1] S. Mori et al., Protein Expr. Purif. 69(1), 99 (2010).

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