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Crystal structure of a novel diadenosine 5′,5‴-P^1,P^4-tetraphosphate phosphorylase from *Mycobacterium tuberculosis* H37Rv

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Introduction

Rv2613c from *Mycobacterium tuberculosis* H37Rv is a novel 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-φ-H-φ-H-φ-φ, where φ is a hydrophobic amino acid. This feature is quite unique among Ap_4A phosphorylases because the HIT motif has been reported to be the characteristic structure of HIT family diadenosine polyphosphate (Ap_nA) hydrolases. Instead of HIT motif, typical Ap_4A phosphorylases usually contain H-X-H-X-Q motif. Furthermore, the amino acid sequence of Rv2613c is more homologous to that of HIT family Ap_nA hydrolases than to that of typical Ap_4A phosphorylases. These observations indicate that Rv2613c is a unique Ap_4A phosphorylase with a primary structure similarity to that of hydrolases rather than phosphorylases. In order to analyze the more detailed structure-function relationship of Rv2613c, elucidation of the crystal structure is required. Recently, we reported the crystallization and preliminary X-ray analysis of Rv2613c [2]. Here, we describe the 1.89 Å resolution crystal structure of Rv2613c.

Methods

We crystallized Rv2613c and collected the diffraction data as described previously [2]. Briefly, Selenomethionine (SeMet)-Rv2613c was crystallized by hanging drop vapor diffusion. The diffraction data for SeMet-Rv2613c were collected at the AR-NW12A station of the Photon Factory at a wavelength of 0.97901 Å. The crystal structure of Rv2613c was solved by using single-wavelength anomalous dispersion (SAD).

Results

Previously, we showed that the Rv2613c crystal belongs to the *C*2 space group and has unit cell parameters of a = 101.5 Å, b = 63.6 Å, c = 79.1 Å, and β = 110.9˚[2]. In addition, we reported that Rv2613c exists as a homotetramer of 25 kDa subunits in solution [1] and that there are 2 subunits per asymmetric unit, which are characterized by a V_M of 2.41 $Å^3 \cdot Da^{-1}$ and a solvent content of 49.1% [2]. Here, we solved the crystal structure of Rv2613c at 1.89 Å resolution. The final R-factor and R_{free} were 17.6% and 20.1%, respectively. The final model of Rv2613c consisted of 325 residues, 233 water molecules, 3 phosphate ions, and 2 tetraethylene glycols in the asymmetric unit, which included subunits A and B (Rv2613c-A/-B) (Fig. 1). Electron density was present for all residues in Rv2613c-A, except for the His tag and residues 1–13, 36–54, and 171–175. Similarly, electron density was present for all residues in Rv2613c-B, except for the His tag and residues 1–23. In addition, residues 196–198 in Rv2613c-B were derived from the expression vector. The average B-factors for all atoms of Rv2613c-A, Rv2613c-B, water molecules, phosphate ions, and tetraethylene glycols were 32.7, 26.5, 36.3, 36.6, and 43.8 $Å^2$, respectively. Ramachandran plot analysis showed that the φ/ψ angle pairs of most residues (322/325; 99.1%) were in the favored regions, whereas the pairs of the remaining residues (3/325; 0.9%) were in the allowed regions. The r.m.s. deviation from ideality is 0.005 Å for bond distances and 1.025 degrees for bond angles.

PDB accession code: 3ANO

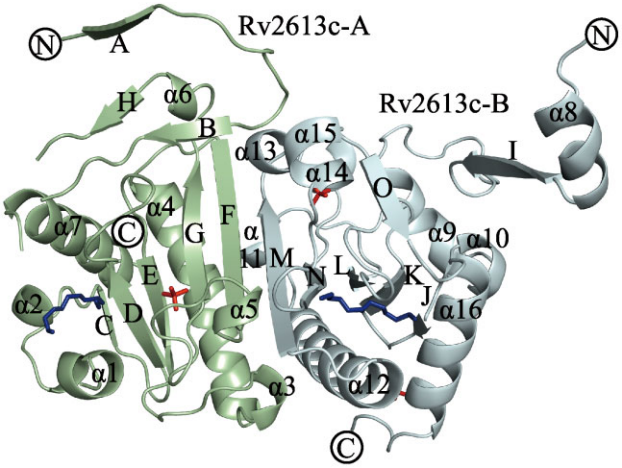

Fig. 1. Ribbon diagram of Rv2613c subunits A (green) and B (cyan) (Rv2613c-A/-B) in the asymmetric unit. The N- and C-termini are indicated by circled letters. The α helices are numbered and β strands are lettered in order from the N-terminus of Rv2613c-A to the C-terminus of Rv2613c-B. Phosphate ions and tetraethylene glycols are modeled as red and blue sticks, respectively.

References

[1] S. Mori et al., Protein Expr. Purif. 69(1), 99 (2010).
[2] S. Mori et al., Acta Cryst. F 66(3), 279 (2010).

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