BL-5A, BL-17A, AR-NW12A/2014G596

Crystallographic analysis of diadenosine tetraphosphate phosphorylase from *Mycobacterium tuberculosis* H37Rv complexed with ADP

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1 Introduction

Recently, we found that Rv2613c protein of *Mycobacterium tuberculosis* H37Rv was a novel diadenosine tetraphosphate (Ap_4A) phosphorylase and determined its crystal structure [1,2]. Results of structure–function analyses suggested that *M. tuberculosis* Ap_4A phosphorylase had a unique substrate-binding site. In the present study, we obtained crystals of *M. tuberculosis* Ap_4A phosphorylase complexed with ADP to assess its substrate-binding site in detail.

2 Experiment

M. tuberculosis Ap_4A phosphorylase was expressed and purified, as described previously [1]. Next, *M. tuberculosis* Ap_4A phosphorylase complexed with ADP was crystallized by performing a hanging drop vapor diffusion method in a 24-well plate. Briefly, 5 μL protein solution (10 $mg \cdot mL^{-1}$) containing ADP (0.1 mM) was mixed with 5 μL crystallization solution (0.1 M sodium cacodylate [pH 6.3], 0.2 M lithium sulfate, and 28.5% polyethylene glycol 400). A drop of this mixture was suspended on a siliconized coverslip over 0.6 mL crystallization solution. Prismatic colorless crystals of *M. tuberculosis* Ap_4A phosphorylase complexed with ADP developed after approximately 2 weeks of incubating the drop at 20°C and grew to a maximum size of 0.3 mm. Diffraction data of *M. tuberculosis* Ap_4A phosphorylase complexed with ADP were collected at BL-5A, BL-17A, and AR-NW12A stations. The crystals were harvested using a cryoloop, were flash-cooled to 100 K under nitrogen stream, and were analyzed. Next, 360 consecutive images were collected using an oscillation range of 1°, exposure time of 2 s, and wavelength of 1.0000 Å. The diffraction data were processed, merged, and scaled using HKL2000 (DENZO and SCALEPACK) software package. Values of Wilson B-factor, Matthew's coefficient, and solvent content were calculated using CCP4 program.

3 Results and Discussion

The diffraction data of crystals of *M. tuberculosis* Ap_4A phosphorylase complexed with ADP were collected using synchrotron radiation. A single crystal resulted in a diffraction of 3.2 Å. Preliminary characterization of the crystal indicated that it belonged to monoclinic space group *C*2, with unit cell parameters of a = 101.7, b = 63.4, c = 79.3 Å, and β= 111.2°. Furthermore, 7,462 independent reflections were obtained using an R_{merge} of 15.9%. Data collection statistics for the crystal are summarized in Table 1.

M. tuberculosis Ap_4A phosphorylase formed a homotetramer of 25-kDa subunits in solution [1,2]. One asymmetric unit of *M. tuberculosis* Ap_4A phosphorylase included two subunits, with a Matthew's coefficient of 2.38 $Å^3 \cdot Da^{-1}$ and solvent content of 48.43%.

Studies assessing the structure of *M. tuberculosis* Ap_4A phosphorylase complexed with ADP by using molecular replacement method are in progress. Coordinates of the structure of wild-type *M. tuberculosis* Ap_4A phosphorylase (PDB code: 3ANO) were used as a search model in the present study.

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)	101.7, 63.4, 79.3
(*β*) (deg)	111.2
Resolution range (Å)	73.94–3.20 (3.37–3.20) [a]
Total No. of reflections	49961 (7247)
No. of Unique reflections	7462 (1068)
Redundancy	6.7 (6.8)
Completeness (%)	95.1 (94.9)
Mean $I/\sigma(I)$	10.0 (4.5)
R_{merge} [b]	15.9 (40.2)
Wilson B-factor (Å)	24.4

[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.

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References

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