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Crystallization and preliminary X-ray diffraction analysis of MJ0458, an adenylate kinase from *Methanocaldococcus jannaschii*

Adenylate kinase plays a very important role in regulating adenylate species in the cell. *Methanocaldococcus jannaschii* is a rich resource of unique enzymes. Here, MJ0458, an adenylate kinase from *M. jannaschii*, was crystallized. A set of X-ray diffraction data to 2.70 Å resolution was collected on beamline BL-17U of the Shanghai Synchrotron Radiation Facility (SSRF). The crystal belonged to space group $P4_12_12$ or $P4_32_12$. The unit-cell parameters were $a = b = 76.18$, $c = 238.70$ Å, $\alpha = \beta = \gamma = 90^\circ$.

1. Introduction

Adenylate kinase (AK; EC 2.7.4.3) is a small monomeric phosphoryltransferase that catalyzes the reversible transfer of a phosphoryl group from adenosine triphosphate (ATP) to adenosine monophosphate (AMP) by nucleophilic attack on the γ -phosphate of ATP and plays an important role in cellular energy homeostasis. The net reaction is schematized as $\text{Mg}^{2+}\text{-ATP} + \text{AMP} \rightleftharpoons \text{Mg}^{2+}\text{-ADP} + \text{ADP}$ (Walsh, 1979). The importance of AK is reflected in its ubiquitous presence in many different organisms and tissues. There are two oligomeric classes of AK in microorganisms: one is monomeric and the other is trimeric (Davlieva & Shamoo, 2010).

Methanocaldococcus jannaschii is a thermophilic methanogenic archaeon in the class Methanococci. It was the first archaeon to have its complete genome sequenced (Bult *et al.*, 1996). Many novel metabolic pathways have been elucidated in *M. jannaschii*. It is a rich resource of unique enzymes and biosynthetic pathways relating to the biosynthesis of methanogenic cofactors (Haase *et al.*, 2003).

The MJ0458 protein in *M. jannaschii* was predicted to be related to aspartokinase and uridylylate kinase. However, its function showed that it was an adenylate kinase (Grochowski *et al.*, 2012). Therefore, the structure of the MJ0458 protein might provide some clues to its exact function and its evolutionary relationship to aspartokinase and uridylylate kinase. In this report, we describe the crystallization and preliminary X-ray diffraction analysis of the protein of MJ0458.

2. Materials and methods

2.1. Cloning, expression and purification

The DNA of full-length MJ0458 was obtained from the *M. jannaschii* genome by PCR using the primers 5'-GGAATTCCA-TATGCATATAGTAAAAATTGG-3' (forward) and 5'-CCGCTC-GAGTTATATTTTATCTATTCC-3' (reverse) (Invitrogen). The PCR product was digested by the restriction endonucleases *Nde*I and *Xho*I and then inserted into pET-28a vector (Novagen) with a His₆ tag at the N-terminus. The plasmid was then transformed into the *Escherichia coli* BL21(DE3) expression strain (Novagen). The cells were cultured in LB medium with 50 mg l⁻¹ kanamycin until the OD₆₀₀ reached 0.8 and were then induced with 0.25 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) at 289 K for a further 20 h. The cells were harvested by centrifugation, resuspended in buffer consisting of 20 mM Tris-HCl pH 7.5, 200 mM NaCl and lysed by sonication. The soluble portion obtained by centrifugation at 14 000g for 30 min was

Table 1

Data-collection statistics.

Values in parentheses are for the outermost resolution shell.

Space group	$P4_12_12$ or $P4_32_12$
Wavelength (Å)	0.97915
Unit-cell parameters (Å, °)	$a = b = 76.18$, $c = 238.70$, $\alpha = \beta = \gamma = 90$
Resolution (Å)	50.00–2.70 (2.75–2.70)
No. of observed reflections	174726 (8651)
No. of unique reflections	20142 (972)
Multiplicity	8.7 (8.9)
Completeness (%)	99.7 (100)
Mean $I/\sigma(I)$	46.5 (7.7)
$R_{\text{merge}}^{\dagger}$	0.064 (0.398)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of reflection hkl and $\langle I(hkl) \rangle$ is the mean intensity of reflection hkl .

applied onto an Ni-NTA column (GE Healthcare, USA) pre-equilibrated with buffer consisting of 20 mM Tris-HCl pH 7.5, 200 mM NaCl. The protein was eluted using a gradient from buffer A (20 mM Tris-HCl pH 7.5, 200 mM NaCl) to buffer B (20 mM Tris-HCl pH 7.5, 200 mM NaCl, 300 mM imidazole). Final purification was achieved on a HiLoad 16/60 Superdex 200 column (GE Healthcare, USA) pre-equilibrated with buffer A. The protein purity was determined by 15% SDS-PAGE stained with Coomassie Brilliant Blue. The protein concentration was determined using the BCA Protein Assay Kit (Pierce, USA). The MJ0458 protein was stored at 193 K until use.

2.2. Crystallization

The MJ0458 protein (stored at 193 K) was thawed on ice. The concentration of the protein was 10 mg ml^{-1} in 20 mM Tris-HCl pH 7.5, 200 mM NaCl. Initial screening experiments were performed using Crystal Screen and Crystal Screen 2 (Hampton Research). The protein was crystallized using the hanging-drop vapour-diffusion method. 1 μl protein solution was added to 1 μl reservoir solution at 187 K. Diffraction-quality crystals were obtained after 1 week in condition No. 9 of Crystal Screen 2 (0.1 M sodium acetate trihydrate, 2.0 M sodium chloride pH 4.6).

Figure 1

SDS-PAGE analysis of the MJ0458 protein. The protein was analyzed on 15% SDS-PAGE stained using Coomassie Brilliant Blue. Left lane, MJ0458 protein (24 kDa); right lane, molecular-mass markers (labelled in kDa).

2.3. X-ray diffraction data collection and processing

For data collection, the crystals were transferred into cryoprotectant solution consisting of 0.1 M sodium acetate trihydrate, 2.0 M sodium chloride, 25% (v/v) glycerol and were then cooled in liquid nitrogen. A set of X-ray diffraction data for the MJ0458 protein was collected using a Jupiter CCD detector at 100 K on beamline BL-17U of the Shanghai Synchrotron Radiation Facility (SSRF). The diffraction data set was processed using HKL-2000 (Otwinowski & Minor, 1997).

3. Results and discussion

Full-length recombinant MJ0458 protein with a His₆ tag at the N-terminus was expressed and purified (Fig. 1). The protein was crystallized in basic buffer (pH 4.6) consisting of 0.1 M sodium acetate

Figure 2

Crystal of recombinant MJ0458 protein from a condition consisting of 0.1 M sodium acetate trihydrate, 2.0 M sodium chloride pH 4.6.

Figure 3

X-ray diffraction image from an MJ0458 protein crystal.

trihydrate, 2.0 M sodium chloride (Fig. 2). A single crystal was used for X-ray diffraction experiments and a set of diffraction data was collected to 2.70 Å resolution (Fig. 3). A summary of the data-collection statistics is given in Table 1. The crystal belonged to space group $P4_12_12$ or $P4_32_12$. The unit-cell parameters were $a = b = 76.18$, $c = 238.70$ Å, $\alpha = \beta = \gamma = 90^\circ$. The overall R_{merge} was 6.4% and the R_{merge} in the outermost shell was 39.8%. Matthews coefficients of 3.56 or 2.38 Å³ Da⁻¹ suggest that there are two or three MJ0458 protein molecules in the asymmetric unit (Matthews, 1968). Because of low protein sequence identity (27% to PDB entry 3lkk and 25% to PDB entry 3ll9; Mabanglo *et al.*, 2010), we failed to identify a solution by molecular-replacement trials. Multi-wavelength anomalous diffraction using a selenomethionyl derivative of the protein is under way as a phase-solution strategy.

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