Crystallization and preliminary X-ray diffraction analysis of the Nif3-family protein MJ0927 from *Methanocaldococcus jannaschii*

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Received 7 October 2012
Accepted 1 December 2012

MJ0927 is a member of the Nif3 family and is widely distributed across living organisms. Although several crystal structures of Nif3 proteins have been reported, structural information on archaeal Nif3 is still limited. To understand the structural differences between bacterial and archaeal Nif3 proteins, MJ0927 from *Methanocaldococcus jannaschii* was purified and crystallized using the sitting-drop vapour-diffusion method. The crystals diffracted to a resolution of 2.47 Å and belonged to the orthorhombic space group *C*222, with unit-cell parameters *a* = 81.21, *b* = 172.94, *c* = 147.42 Å. Determination of this structure may provide insights into the function of MJ0927.

1. Introduction

As worldwide projects have made a large number of genome sequences available, the number of hypothetical proteins with unknown function is steadily increasing. To elucidate the possible function of these hypothetical proteins, it is necessary to study their three-dimensional structure. Structural genome projects around the world provide templates useful for modelling tens of thousands of protein sequences and inferring their functions from a structural perspective (Xiao *et al.*, 2010). New protein structures can further provide crucial insights into unrecognized evolutionary relationships. NGG1p-interacting factor 3 (Nif3)-like protein is considered as a high-priority target since this conserved hypothetical protein is widely distributed in all kingdoms of living organisms and is involved in transcriptional regulation (Galperin & Koonin, 2004).

The Nif3-like superfamily was first discovered by Martens and coworkers and was identified in a yeast two-hybrid screen as an NGG1p-interacting protein (Martens *et al.*, 1996). Identification and characterization of the human and murine Nif311 proteins showed that only the N- and C-terminal regions of the Nif3 proteins are highly conserved from bacteria to humans (Tascou *et al.*, 2003). Akiyama *et al.* (2003) demonstrated that murine Nif311 can be transported into the nucleus through interactions with Trip15/CSN2, which functions as a transcriptional corepressor. These studies indicate that Nif3 proteins in eukaryotes are involved in transcriptional regulation. However, the biological function of Nif3 proteins in prokaryotes and archaea is currently obscure.

On the basis of sequence analysis, MJ0927 has been classified as a conserved hypothetical protein. It contains 249 amino acids and shares 33% sequence identity with a similar protein from *Streptococcus pneumoniae*, 34% sequence identity with a protein from *Staphylococcus aureus* (Saikatendu *et al.*, 2006), 25% sequence identity with a protein from *Escherichia coli* (Ladner *et al.*, 2003) and 28% sequence identity with a protein from *Thermus thermophilus* (Tomoike *et al.*, 2009). The crystal structures of these Nif3-family proteins comprise two similar α/β domains that resemble a ferredoxin-like structure. However, to date, the structure of archaeal Nif3 is not available. A three-dimensional structural study of MJ0927 would help in understanding structural differences between the archaeal and bacterial Nif3 members. In this study, we present the expression, purification, crystallization and preliminary X-ray crystallographic analysis of MJ0927.

Table 1

Data-collection statistics for the MJ0927 crystal.

Values in parentheses are for the highest resolution shell.

X-ray wavelength (Å)	0.97622
Space group	C222
Unit-cell parameters (Å)	$a = 81.21$, $b = 172.94$, $c = 147.42$
Resolution (Å)	30.0–2.47 (2.56–2.47)
No. of measured reflections	271300 (26582)
No. of unique reflections	37448 (3692)
Multiplicity	7.2 (7.2)
$R_{\text{merge}}^{\dagger}$ (%)	5.3 (46.5)
Data completeness (%)	99.9 (100.0)
$\langle I/\sigma(I) \rangle$	34.3 (4.6)

$$\dagger R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}.$$

2. Materials and methods

2.1. Cloning, expression and purification

The 747-bp open reading frame encoding MJ0927 was PCR amplified using the *Methanocaldococcus jannaschii* genomic DNA as template. It was then cloned into the pET24b(+) vector (Novagen) for the His₆-tagged MJ0927 protein expression in *E. coli* BL21 (DE3) cells, which were grown in 4 l LB medium containing 25 µg ml^{−1} kanamycin at 310 K until the culture density reached an OD₆₀₀ of 1.0. IPTG was then added to a final concentration of 0.5 mM and growth was continued for a further 20 h. Cultured cells were harvested by centrifugation at 1590g for 30 min at 277 K.

The cell pellet was resuspended in buffer A (50 mM Tris–HCl pH 8.0, 500 mM NaCl, 5 mM imidazole) and then disrupted by sonication. The crude lysate was incubated at 338 K for 10 min and kept on ice. The lysate was then centrifuged at 22 300g for 90 min at 277 K. The supernatant was collected and applied onto Ni–NTA His-bind resin (GE Healthcare) pre-equilibrated with buffer A. The protein was eluted with a 0–200 mM linear gradient of imidazole. Fractions containing MJ0927 were identified by SDS–PAGE and dialysed overnight against buffer B (25 mM Tris–HCl pH 8.5, 5 mM NaCl) at 277 K. The dialysed sample was subjected to a HiTrap Q HP column (GE Healthcare) with buffer B for dialysis. After washing the column with the same buffer, the bound proteins were eluted using a linear gradient of 0–1 M NaCl. Fractions containing MJ0927 were concentrated and passed through a HiLoad 16/60 Superdex-200 size-exclusion column (GE Healthcare) equilibrated in buffer C [50 mM Tris–HCl pH 8.0, 100 mM NaCl, 5% glycerol, 2 mM tris(2-carboxy-ethyl)phosphine]. The fractions containing MJ0927 were pooled and concentrated to 35 mg ml^{−1} for crystallization screening.

2.2. Crystallization

Initial crystallization screening of MJ0927 was performed at 277 K with commercially available kits from Hampton Research (Index, PEG/Ion, PEG Rx, Crystal Screen and Crystal Screen 2), Emerald BioSystems (Wizard I–IV and PACT 1–2) and Molecular Dimensions (PGA Screen, Clear Strategy Screen I and II) by the sitting-drop vapour-diffusion technique in 24-well VDX plates (Hampton Research). A total of 1 µl protein solution (35 mg ml^{−1} in buffer C) and 1 µl reservoir solution were mixed and equilibrated against 400 µl reservoir solution at 277 K. After 1 month of incubation, crystals appeared in 24 conditions, but the best diffracting crystals were obtained from the following condition: 0.1 M ammonium sulfate, 0.3 M sodium formate, 0.1 M sodium acetate, 3% PGA-LM, 20% MPD (condition No. 27 of PGA Screen from Molecular Dimensions). The crystals grew to final dimensions of 0.2 × 0.1 × 0.1 mm after 30 d.

2.3. Data collection

Diffraction data were collected at 100 K on the BL13C1 beamline at the National Synchrotron Radiation Research Center (NSRRC), Taiwan. Crystals of MJ0927 were soaked in reservoir solution supplemented with 25% glycerol for 15 s and flash-cooled in liquid nitrogen. A total of 180 images were recorded using an ADSC Quantum 315R CCD detector. The data were collected in 1° oscillation steps over a range of 180° with a 300 mm sample-to-detector distance and an exposure time of 10 s. The diffraction data were indexed and integrated using the *HKL-2000* processing software (Otwinowski & Minor, 1997). Crystal parameters and data-collection statistics are summarized in Table 1.

3. Results and discussion

The MJ0927 gene was successfully cloned into the pET24b(+) expression vector as verified by DNA sequencing. The protein was overexpressed in *E. coli* BL21 (DE3) in a soluble form as a fused

Figure 1

SDS–PAGE of purified MJ0927. Lane M, molecular-weight markers (labelled in kDa); lane 1, MJ0927 purified with an Ni–NTA column; lane 2, MJ0927 purified with a HiTrap Q column; lane 3, MJ0927 purified by gel filtration.

Figure 2

Crystallization of MJ0927 from *M. jannaschii*. The approximate dimensions of the crystals were 0.2 × 0.1 × 0.1 mm.

Figure 3

Diffraction pattern of MJ0927 collected on the NSRR BL13C1 beamline from a crystal flash-cooled in 25% glycerol.

protein with an extra octapeptide LEH₆ at the C-terminal end. MJ0927 protein was purified to homogeneity and its purity was checked using SDS-PAGE (Fig. 1). The typical yield of the protein was about 30 mg protein per litre of cell-culture medium.

The purified protein yielded many hits from initial screenings. Plate-like crystals with various forms were obtained in sodium acetate and several ammonium salts such as ammonium sulfate and phosphate. Long needles also grew from PEG 3350 and PEG 6000 solutions at various pH values. After optimization was performed by varying the protein and precipitant, the best crystals were obtained in a buffer consisting of 0.1 M ammonium sulfate, 0.3 M sodium formate, 0.1 M sodium acetate, 3% PGA-LM, 20% MPD. The dimensions of the crystals used for data collection were approximately 0.2 × 0.1 × 0.1 mm and they diffracted to a maximum resolution of 2.47 Å (Figs. 2 and 3). The preliminary crystallographic analysis indicated that the crystals belonged to space group C222, with unit-cell parameters $a = 81.21$, $b = 172.94$, $c = 147.42$ Å. The data-collection and processing statistics are summarized in Table 1. Based on Matthews coefficient calculations, three (58.8% solvent content) or four (45.0% solvent content) molecules could be accommodated in the asymmetric unit, with an acceptable V_M in the range 2.24–2.98 Å³ Da^{−1} (Matthews, 1968). Molecular-replacement trials using several structural models of Nif3-family proteins failed because the existing models had relatively low sequence similarity. Structure

determination using anomalous dispersion of native ions or other substituted metal ions is currently under way.

We thank the National Synchrotron Radiation Research Center (NSRR, Taiwan) for assistance during data collection. We acknowledge Dr Shan-Ho Chou for thoughtful discussions regarding the manuscript. This work was supported by grants from the National Science Council (grant Nos. NSC99-2313-B-241-001 and NSC100-2313-B-241-006 to YC).

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