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Cloning, purification, crystallization and preliminary X-ray crystallographic analysis of the N-terminal domain of DEAD-box RNA helicase from *Staphylococcus aureus* strain Mu50

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DEAD-box helicases are enzymes with an ATP-dependent RNA-unwinding function that are involved in a variety of cellular processes including RNA splicing, ribosome biogenesis and RNA degradation. In this study, the N-terminal domain of DEAD-box RNA helicase from *Staphylococcus aureus* strain Mu50 was overexpressed in *Escherichia coli*, purified and crystallized. Diffraction data were collected to 2.60 Å resolution using a synchrotron-radiation source. The crystal belonged to space group *P*1, with unit-cell parameters $a = 70.81$, $b = 80.23$, $c = 86.25$ Å, $\alpha = 69.54$, $\beta = 66.54$, $\gamma = 87.32^\circ$. The unit cell contained six molecules, with a corresponding V_M of 2.91 Å^3 Da^{-1} and a solvent content of 56.1%.

1. Introduction

Most processes involving RNA and DNA metabolism require the unwinding and alteration of the higher order structures of nucleic acids. Helicases are enzymes that couple the energy of ATP hydrolysis to the unwinding of nucleic acids. DEAD-box proteins, which were named after the strictly conserved sequence Asp-Glu-Ala-Asp, are widely found from bacteria to humans and constitute the largest RNA-helicase family (Linder *et al.*, 1989). DEAD-box proteins are involved in many RNA metabolic processes, including RNA splicing, ribosome biogenesis and RNA degradation (Mohr *et al.*, 2002; Venema & Tollervey, 1995; Rocak & Linder, 2004). Based on conserved sequence motifs, helicases have been divided into three superfamilies: SF1–3 (Gorbalenya & Koonin, 1993). DEAD-box proteins belong to SF2. The minimal core of SF2 proteins is composed of two RecA-like domains, an N-terminal RecA-like domain (NTD) and a C-terminal RecA-like domain (CTD), which are connected by a flexible linker (Caruthers & McKay, 2002). The NTD contains most of the conserved motifs involved in nucleotide binding, such as the Q-motif, the GKT motif (Walker A P-loop), the DEAD box and the SAT motif (Walker *et al.*, 1982; Pause & Sonenberg, 1992; Tanner *et al.*, 2003). To date, the structures of several prokaryotic and eukaryotic DEAD-box proteins have been determined. In these structures the NTD and the CTD have conserved folds, but the conformations of the loops in the domain structures differ from each other and the interdomain orientations are strikingly diverse (Sengoku *et al.*, 2006). To date, structural information on prokaryotic DEAD-box proteins is limited to the enzymes from hyperthermophiles, including *Thermus thermophilus*, *Bacillus stearothermophilus* and *Methanococcus jannaschii* (Rudolph *et al.*, 2006; Carmel & Matthews, 2004; Story *et al.*, 2001). However, no structure of a DEAD-box protein from a mesophilic bacterium has yet been determined.

To provide further structural data with regard to the catalytic mechanism of DEAD-box helicases and the structural characteristics of the DEAD-box proteins of mesophilic bacteria, we have crystallized and performed X-ray crystallographic experiments on the N-terminal domain of the DEAD-box helicase from *Staphylococcus aureus* strain Mu50.

2. Materials and methods

2.1. Cloning, protein expression and purification

The gene encoding the N-terminal domain (amino acids 1–212) of DEAD-box helicase was amplified from genomic DNA of *S. aureus* strain Mu50 by the polymerase chain reaction (PCR) using specific primers. The forward primer contained an *Nde*I restriction site (bold) and had the sequence 5′-CCC **CAT ATG** GCA AAA CAT CCA TTC G-3′, while the reverse primer contained a *Xho*I site (bold) and had the sequence 5′-CCC **CTC GAG** TTT TTT ACT GTC GAC AGC TAC ATA T-3′. The PCR product was then subcloned between the *Nde*I and *Xho*I sites of pET-22b vector (Novagen, USA). This construct contains an additional hexahistidine tag (LEHHHHHH) at the C-terminus for purification purposes. The recombinant plasmid was transformed into *Escherichia coli* strain BL21 (DE3) (Novagen) and the cells were grown in a shaking incubator at 310 K in LB broth medium supplemented with 50 µg ml^{-1} ampicillin. Protein expression was induced by adding 0.5 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) when the cells reached an optical density at 600 nm of about 0.6 and the cells were cultured at the same temperature for an additional 4 h. The cultured cells were harvested by centrifugation at 3000*g* for 30 min at 277 K. The cell pellet was resuspended in binding buffer (20 m*M* Tris pH 8.0, 10% glycerol, 200 m*M* NaCl and 20 m*M* imidazole) and disrupted by sonication at 277 K. The crude lysate was centrifuged at 25 000*g* for 1 h at 277 K. The supernatant was then loaded onto an Ni^{2+}-chelating HisTrap FF crude column (GE Healthcare, USA) which had been pre-equilibrated with binding buffer. The protein was eluted with elution buffer (20 m*M* Tris pH 8.0, 10% glycerol, 200 m*M* NaCl and 400 m*M* imidazole). The eluted protein was concentrated and further purified by size-exclusion chromatography on a Superdex 200 16/60 column (GE Healthcare, USA) which had been pre-equilibrated with buffer consisting of 20 m*M* Tris pH 8.0, 10% glycerol and 200 m*M* NaCl. The purified protein was concentrated to 10 mg ml^{-1} in the size-exclusion buffer and the purity of the protein was examined by 12% SDS–PAGE and determined to be >95%.

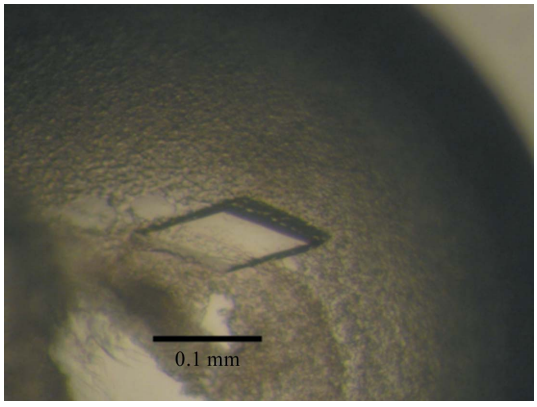

Figure 1
A crystal of the N-terminal domain of the DEAD-box helicase from *S. aureus* strain Mu50 grown using 0.1 *M* Tris pH 7.5, 0.2 *M* magnesium chloride and 20% PEG 3350.

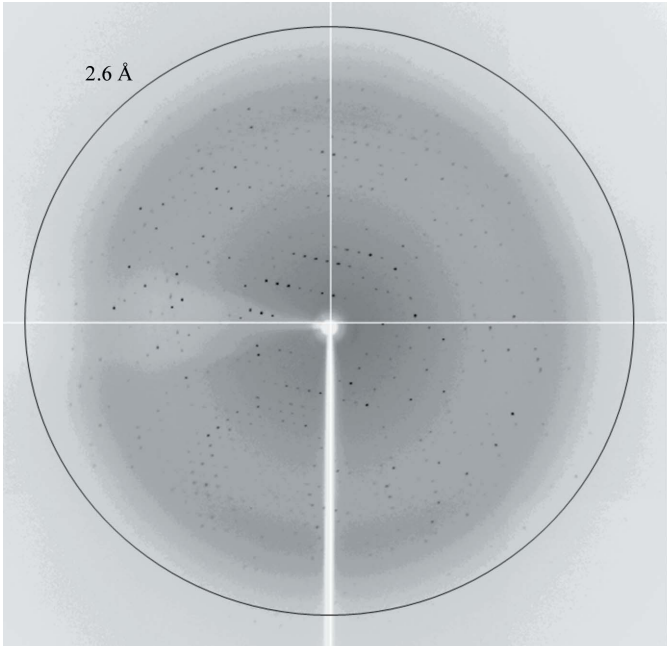

Figure 2
Typical diffraction image of the crystal of the N-terminal domain of DEAD-box helicase. The resolution limit is indicated as a circle with its value (2.6 Å).

2.2. Crystallization and data collection

Crystallization of the protein was initiated by crystal screening at 293 K using the hanging-drop vapour-diffusion method in 24-well VDX plates (Hampton Research, USA) with drops consisting of 1 µl protein solution in size-exclusion buffer and 1 µl well solution equilibrated against 500 µl well solution. Commercial screening kits from Hampton Research and Emerald BioSystems (Crystal Screen, Crystal Screen 2, Index, SaltRx and Wizard I and II) were used for preliminary screening. Initial crystals were obtained using the following condition: 0.1 *M* Tris pH 7.0, 0.2 *M* calcium acetate, 20% PEG 3000. The crystallization condition was further optimized to obtain better crystals; the drops used in the optimized crystallization condition were prepared by mixing 1.0 µl protein solution with 1.0 µl reservoir solution (0.1 *M* Tris pH 7.5, 0.2 *M* magnesium chloride and 20% PEG 3350) and each hanging drop was equilibrated over 500 µl reservoir solution. Suitable-sized crystals were obtained within 3 d (Fig. 1); they were cryoprotected by soaking them for 3 s in cryoprotectant solution containing 0.1 *M* Tris pH 7.5, 0.2 *M* magnesium chloride, 20% PEG 3350 and 25%(*v*/*v*) ethylene glycol and were flash-frozen in liquid nitrogen. Frozen crystals were mounted on the goniometer in a stream of cold nitrogen gas at 100 K. X-ray diffraction data were collected from a frozen crystal using an ADSC Quantum CCD 210 detector on beamline 6B at Pohang Light Source (PLS), South Korea. A total rotation range of 180° was covered using 1.0° oscillations and 15 s exposure per frame. The wavelength of the synchrotron X-ray beam was 1.00 Å and the crystal-to-detector distance was set to 200 mm. X-ray diffraction data were collected to 2.60 Å resolution (Fig. 2). The data were indexed, integrated, scaled and merged using the *HKL*-2000 software package (Otwinowski & Minor, 1997). The crystal structure was solved by the molecular-replacement (MR) method using the *CNS* package (Brünger *et al.*, 1998) with the structure of a monomer of *B. stearothermophilus* DEAD-box protein (PDB code 1q0u; Carmel & Matthews, 2004) as the search model.

3. Results and discussion

The N-terminal domain (amino acids 1–212) of DEAD-box helicase from *S. aureus* strain Mu50 was cloned, overexpressed, purified and crystallized for structural studies. X-ray diffraction data from the crystal indicated that the crystal belonged to space group *P*1, with

Table 1
Data-collection statistics.

Values in parentheses are for the last resolution shell.

Synchrotron	PLS beamline 6B
Wavelength (Å)	1.00
Resolution range (Å)	50.0–2.60 (2.64–2.60)
Space group	*P*1
Unit-cell parameters (Å, °)	a = 70.81, b = 80.23, c = 86.25, α = 69.54, β = 66.54, γ = 87.32
No. of unique reflections	46215
Multiplicity	1.9 (1.7)
Completeness (%)	93.8 (73.4)
Molecules per asymmetric unit	6
V_M (Å^3 Da^{-1})	2.91
Solvent content (%)	56.1
Average $I/\sigma(I)$	15.2 (2.1)
R_{merge}† (%)	6.9 (32.9)

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

unit-cell parameters a = 70.81, b = 80.23, c = 86.25 Å, α = 69.54, β = 66.54, γ = 87.32°. Data-collection statistics are presented in Table 1. The Matthews coefficient suggests the presence of six molecules in the crystallographic asymmetric unit, with a V_M of 2.91 Å^3 Da^{-1} and a solvent content of 56.1% (Matthews, 1968). The structure solution obtained by the molecular-replacement method made it clear that the unit cell contained six protein molecules. The best molecular-replacement solution showed good crystal packing, giving an R_{work} of 43.7% (R_{free} = 45.2%) for data in the resolution range 20–3.5 Å. The other solutions had R factors of over 50%. In the unit cell, no fold or screw axes exist among the six molecules and each pair of molecules has very similar but slightly tilted orientations. Owing to the slight discrepancy in the molecular orientation between the pairs of molecules, it was impossible to index the reflections with smaller unit-cell parameters. The final model is currently being refined and structural details will be described in a separate paper.

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References

Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.
Carmel, A. B. & Matthews, B. W. (2004). *RNA*, **10**, 66–74.
Caruthers, J. M. & McKay, D. B. (2002). *Curr. Opin. Struct. Biol.* **12**, 123–133.
Gorbalenya, A. E. & Koonin, E. V. (1993). *Curr. Opin. Struct. Biol.* **3**, 419–429.
Linder, P., Lasko, P. F., Ashburner, M., Leroy, P., Nielsen, P. J., Nishi, K., Schnier, J. & Slonimski, P. P. (1989). *Nature (London)*, **337**, 121–122.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mohr, S., Stryker, J. M. & Lambowitz, A. M. (2002). *Cell*, **109**, 769–779.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **277**, 307–326.
Pause, A. & Sonenberg, N. (1992). *EMBO J.* **11**, 2643–2654.
Rocak, S. & Linder, P. (2004). *Nature Rev. Mol. Cell Biol.* **5**, 232–241.
Rudolph, M. G., Heissmann, R., Wittmann, J. G. & Klostermeier, D. (2006). *J. Mol. Biol.* **361**, 731–743.
Sengoku, T., Nureki, O., Nakamura, A., Kobayashi, S. & Yokoyama, S. (2006). *Cell*, **125**, 287–300.
Story, R. M., Li, H. & Abelson, J. N. (2001). *Proc. Natl Acad. Sci. USA*, **98**, 1465–1470.
Tanner, N. K., Cordin, O., Banroques, J., Doère, M. & Linder, P. (2003). *Mol. Cell*, **11**, 127–138.
Venema, J. & Tollervey, D. (1995). *Yeast*, **11**, 1629–1650.
Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. (1982). *EMBO J.* **1**, 945–951