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Crystallization and preliminary X-ray crystallographic analysis of the short-chain dehydrogenase/reductase-type DDB_G0291732 protein from *Dictyostelium discoideum*

Min-Kyu Kim, Hyung-Soon Yim* and Sa-Ouk Kang*

Laboratory of Biophysics, School of Biological Sciences and Institute of Microbiology, Seoul National University, Seoul 151-742, Republic of Korea

Correspondence e-mail: wuseok@hotmail.com, kangsaou@snu.ac.kr

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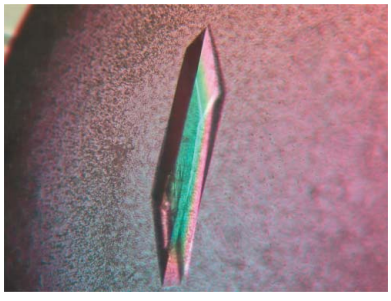
The DDB_G0291732 gene product from *Dictyostelium discoideum*, which is a NmrA-like protein that belongs to the short-chain dehydrogenase/reductase superfamily but shows deviations in conserved sequence regions, has been crystallized by the hanging-drop vapour-diffusion method at 295 K. A 1.65 Å resolution data set was collected using synchrotron radiation. The crystals of DDB_G0291732 protein belonged to space group $P2_1$, with unit-cell parameters $a = 38.5$, $b = 63.7$, $c = 56.0$ Å, $\beta = 91.7^\circ$. Assuming the presence of one molecule in the asymmetric unit, the solvent content was estimated to be about 38.1%.

1. Introduction

The short-chain dehydrogenase/reductase (SDR) superfamily is one of the largest protein superfamilies and is found in all forms of life. SDR-superfamily proteins constitute a large family of NAD(P)(H)-dependent oxidoreductases that share sequence motifs and display similar mechanisms (Persson *et al.*, 2009). Currently, the SDR superfamily has more than 47 000 primary structures available in sequence databases and over 300 crystal structures deposited in the Protein Data Bank. The three-dimensional structures are clearly homologous, with a single-domain globular Rossmann fold consisting of a β-sheet sandwiched between three α-helices on each side. The active site is formed by a triad/tetrad of highly conserved Tyr, Lys, Ser (and Asn) residues (Kavanagh *et al.*, 2008; Filling *et al.*, 2002).

The SDR superfamily can be divided into five subfamilies, with the two major families being the classical and extended SDRs, which are distinguished by the size of the substrate-binding C-terminal domain and the conserved sequence of the cofactor-binding Gly-rich region (Jörnvall *et al.*, 1995). The three minor types of SDR are denoted 'intermediate', 'divergent' and 'complex' SDRs (Persson *et al.*, 2009). In addition, transcriptional regulators such as fungal NmrA (Stammers *et al.*, 2001), proapoptotic oncogenes such as CC3/Tip30 (El Omari *et al.*, 2005) and prokaryotic halohydrin dehydrogenases (de Jong *et al.*, 2003) have been shown to be structurally related to the SDR superfamily and constitute a separate branch referred to as atypical SDRs.

The *Dictyostelium discoideum* protein DDB_G0291732 consists of 308 amino acids and is clearly related to the NmrA-like SDR superfamily; however, it exists as a monomer in solution (Fig. 1*b*), in contrast to common SDR-type enzymes, which are found to be either homodimers or hometetramers (Kristan *et al.*, 2005; Oppermann *et al.*, 2003). Primary-sequence analysis shows that it lacks the extreme N-terminal cofactor-binding Gly-rich region and that the active-site Tyr residue is changed to a His residue, suggesting that it may not have dehydrogenase/reductase activity but may play other physiological role(s) similar to those of the dinucleotide-sensing NmrA or HSCARG (Stammers *et al.*, 2001; Zheng *et al.*, 2007). A better understanding of the biological functions of DDB_G0291732 will require determination of the high-resolution structure of the full-length protein. As a first step towards this goal, we report the overexpression, purification and crystallization of the full-length DDB_G0291732 protein from *D. discoideum* and its preliminary X-ray characterization.



2. Materials and methods

2.1. Expression and purification of the DDB_G0291732 gene product

The DDB_G0291732 gene was amplified by polymerase chain reaction using *D. discoideum* cDNA as a template. The gene was inserted downstream of the T7 promoter of the expression plasmid pET-3a (Novagen) and the resulting construct expressed residues 1–308 of the DDB_G0291732 protein without any additional residues. After verifying the DNA sequence, the plasmid DNA was transformed into *Escherichia coli* strain BL21 (DE3). The cells were grown to an OD_{600} of approximately 0.6 in Luria–Bertani medium containing 0.1 mg ml^{-1} ampicillin (Duchefa) at 310 K and expression was induced with 1 m*M* isopropyl β-D-1-thiogalactopyranoside (Duchefa).

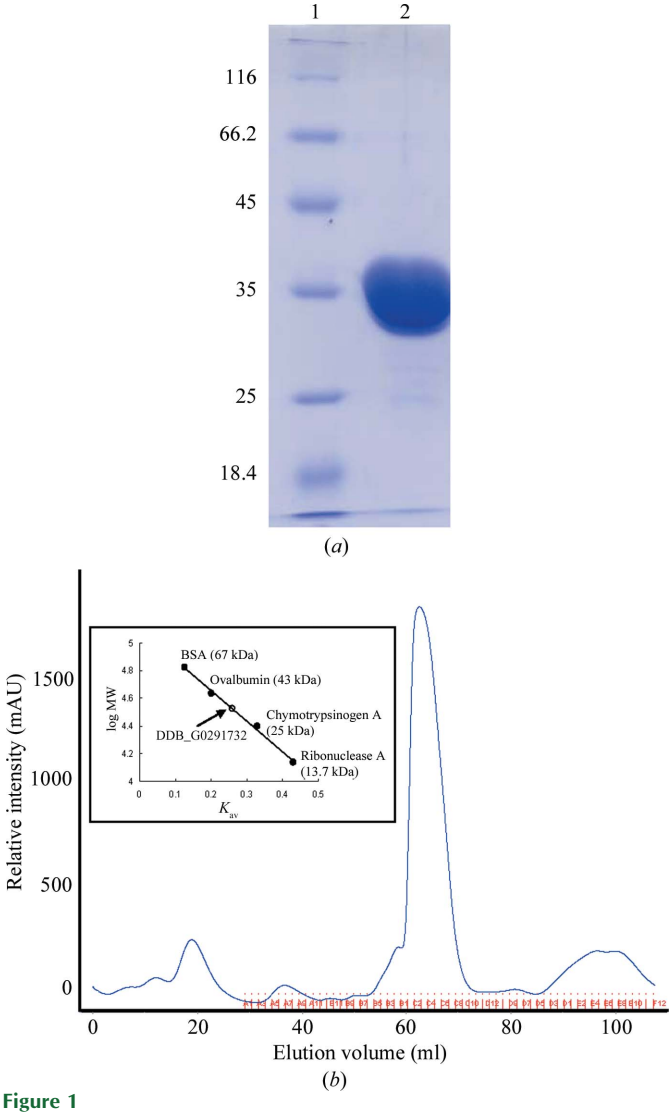


Figure 1
Purification of *D. discoideum* DDB_G0291732 protein. (*a*) 10 µg purified recombinant DDB_G0291732 protein (lane 2) on 12% SDS–PAGE. Lane 1 contains molecular-weight markers (labelled in kDa). (*b*) Gel-filtration chromatography of DDB_G0291732 protein. A single peak was observed that was estimated to correspond to the size of a monomer using protein standards. The inset shows K_{av} against log(molecular weight) for the protein standards (filled circles) and DDB_G0291732 protein (open circle). K_d was calculated from the equation $K_d = (V_e - V_0)/(V_t - V_0)$, where V_e, V_t and V_0 correspond to the eluted volume, the total volume and the void volume, respectively. The standard proteins were BSA (67 kDa), ovalbumin (43 kDa), chymotrypsinogen A (25 kDa) and ribonuclease A (13.7 kDa).

After 12 h induction at 295 K, the cells were harvested and resuspended in 50 m*M* potassium phosphate pH 7.5 (Fluka) containing 0.1 m*M* ethylenediaminetetraacetic acid (EDTA; Fluka). The cells were disrupted by sonication and the cell debris was discarded by centrifugation at 20 000*g* for 30 min. Ammonium sulfate (Fluka) was then added to the supernatant to 55% saturation. After stirring the solution for 1 h, the precipitate was discarded by centrifugation at 20 000*g* for 30 min. The protein solution was loaded onto a Superdex 75 HR 16/60 column pre-equilibrated with 25 m*M* Tris–HCl pH 7.5. The fractions containing an overexpressed 35 kDa band on SDS–PAGE were pooled and concentrated. The proteins were loaded onto a Mono-Q Sepharose column (Amersham Biosciences) and the DDB_G0291732 protein was eluted with washing buffer (25 m*M* Tris–HCl pH 7.5). The purified protein was dialyzed against 25 m*M* Tris–HCl pH 7.5 containing 150 m*M* NaCl and was concentrated to approximately 30 mg ml^{-1} for crystallization trials (Fig. 1*a*).

2.2. Crystallization and X-ray data collection

The DDB_G0291732 protein was crystallized by the hanging-drop vapour-diffusion method using Crystal Screen, Crystal Screen 2, Index, SaltRx, Natrix and Crystal Screen Cryo from Hampton

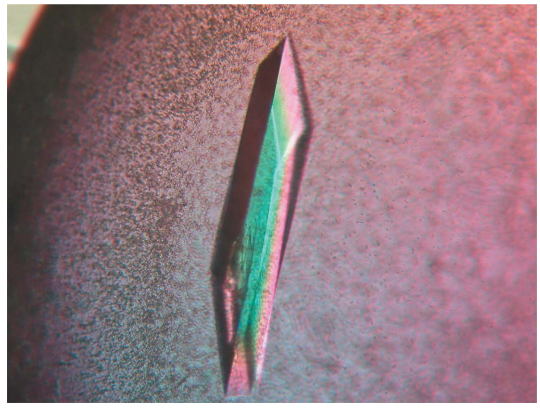


Figure 2
Crystals of DDB_G0291732 protein.

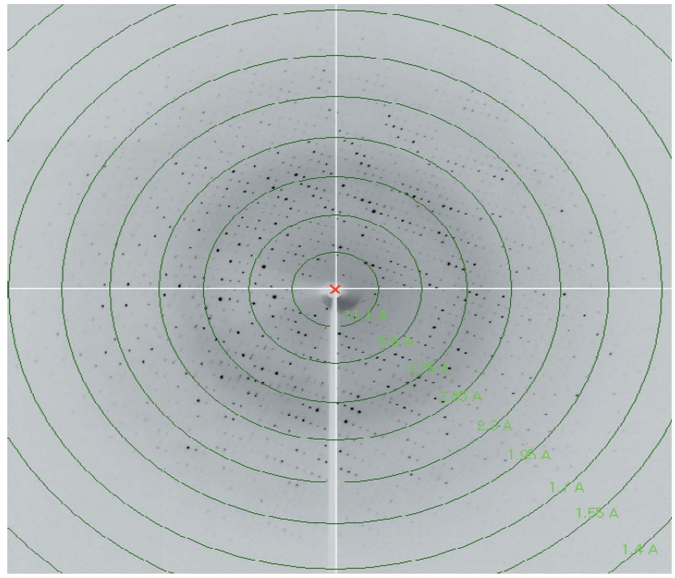


Figure 3
An X-ray diffraction pattern from a crystal of DDB_G0291732 protein.

Table 1
Crystal information and data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 38.5, b = 63.7, c = 56.0, β = 91.7
Wavelength (Å)	1.00000
Resolution (Å)	50–1.65 (1.71–1.65)
Completeness (>0σ) (%)	98.8 (93.3)
$R_{\rm merge}$† (%)	8.6 (23.3)
Average $I/\sigma(I)$	34.2 (3.3)
Unique reflections	32161 (2999)
Average multiplicity	5.7 (3.5)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the mean intensity of symmetry-equivalent reflections.

Research and Wizard I and II from Emerald BioSystems. Droplets consisting of 1.5 µl each of protein solution and reservoir solution were equilibrated against 350 µl reservoir solution at 295 K. Small bundles of rod-shaped crystals were produced in 2 d using 0.2 *M* NaCl, 0.1 *M* bis-tris pH 5.5, 25% PEG 3350. To optimize the crystallization conditions, the pH, salt content and PEG concentration were adjusted. After using Additive Screen (Hampton Research) with the optimized crystallization condition, an enlarged single crystal was obtained using 0.5 *M* $CaCl_2$, 0.1 *M* Tris–HCl pH 6.5, 25% PEG 3350 and 0.1 *M* LiCl (Fig. 2).

15% glycerol was used as a cryoprotectant for data collection under cryogenic conditions. The crystals were soaked in mother liquor containing the cryoprotectant. The crystals were maintained at ∼100 K during data collection in order to minimize radiation damage. A 1.65 Å resolution native data set was collected at 100 K using an Area Detector Systems Corporation (ADSC) Quantum 210 charge-coupled device (CCD) area-detector system on BL-6B of Pohang Light Source (PLS), South Korea (Fig. 3). The exposure time to the synchrotron radiation was 15 s. A total of 360 frames of 1° oscillation were measured with the crystal-to-detector distance set to 150 mm. The diffraction data were processed and scaled using *DENZO* and *SCALEPACK* from the *HKL*-2000 program suite (Otwinowski & Minor, 1997).

3. Results and discussion

The crystal of the DDB_G0291732 protein belonged to the monoclinic space group $P2_1$, with unit-cell parameters a = 38.5, b = 63.7, c = 56.0 Å, β = 91.7°. The crystal volume per unit molecular weight ($V_{\rm M}$) was about 1.99 Å^3 Da^{-1}, with a solvent content of 38.1% by volume (Matthews, 1968), when the asymmetric unit was assumed to contain one molecule. The data-collection statistics are summarized in Table 1.

Solution of the crystal structure of DDB_G0291732 was attempted by molecular replacement with *MOLREP* (Vagin & Teplyakov, 2010) and *Phaser* (McCoy, 2007) using the structures of NmrA (PDB code 1k6i; Stammers *et al.*, 2001), HSCARG (PDB code 2exx; Zheng *et al.*, 2007) and QOR2 (PDB code 2zcu; Kim *et al.*, 2008) as search models. However, all of the trials resulted in failure, indicating that the DDB_G0291732 protein has a different C-terminal domain conformation or cofactor-binding region compared with these proteins. Furthermore, isothermal titration calorimetry data showed that this protein does not interact with any form of NAD(P) (data not shown).

The crystal structure of the NmrA-like DDB_G0291732 protein will provide insight into the diversification of NmrA-like proteins in structure and function. Therefore, we are attempting to grow crystals of selenomethionine-substituted DDB_G0291732 protein in order to solve the crystal structure using the multiple-wavelength anomalous dispersion method.

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