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Crystallization and preliminary X-ray crystallographic study of flavoredoxin from *Desulfovibrio vulgaris* Miyazaki F

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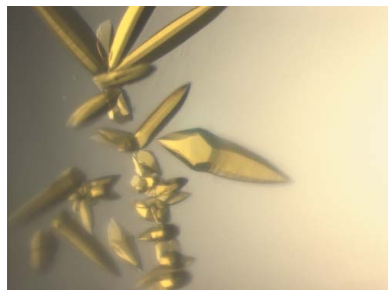

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Flavoredoxin from *Desulfovibrio vulgaris* Miyazaki F has been overexpressed, purified and crystallized using the sitting-drop vapour-diffusion method with 10%(*w*/*v*) PEG 8000, 0.2 *M* zinc acetate and 100 m*M* MES pH 6.0. The diffraction pattern of the crystal extended to 1.05 Å resolution under cryogenic conditions. The space group was determined to be $P3_121$, with unit-cell parameters $a = b = 53.35$, $c = 116.22$ Å. Phase determination was carried out by the SAD method using methylmercuric chloride.

1. Introduction

Desulfovibrio vulgaris is a species of sulfate-reducing bacteria that is known to use sulfate as a terminal electron acceptor in anaerobic respiration. In *Desulfovibrio*, at least three small redox proteins containing flavin mononucleotide have been identified: flavodoxin (Dubourdieu & Le Gall, 1970; Curley *et al.*, 1991), FMN-binding protein (Kitamura *et al.*, 1994) and flavoredoxin (Agostinho *et al.*, 2000). However, their roles in metabolism are still unclear. The structures of flavodoxin (Watenpaugh *et al.*, 1972; Watt *et al.*, 1991; Walsh *et al.*, 1998; Reynolds *et al.*, 2001) and FMN-binding protein have been determined by NMR spectroscopy (Liepinsh *et al.*, 1997) and/or X-ray crystallography (Suto *et al.*, 2000), whereas that of flavoredoxin is still unknown. Recently, we succeeded in cloning a gene encoding flavoredoxin and in constructing an overexpression system using *Escherichia coli* (Takeuchi *et al.*, 2005). Here, we report the crystallization and preliminary crystallographic data of flavoredoxin.

2. Experimental procedures

2.1. Protein expression and purification

The nucleotide sequence and amino-acid sequence data appear in the DDBJ, EMBL and Genbank nucleotide databases under accession Nos. AB214904 and BAD99043, respectively. A high-level expression system using pMK2 vector (Hibino *et al.*, 1994) of flavoredoxin (residues 1–190) from *D. vulgaris* Miyazaki was constructed using a *tac* promoter in *E. coli* (Takeuchi *et al.*, 2005). The transformants were transformed into JM109 and cultured at 331 K until the cell density reached $OD_{600} = 0.6$. IPTG solution (1.0 m*M*) was then added and the transformants were cultured at 303 K for 4 h. The resulting cells were harvested, suspended in 10 m*M* Tris–HCl buffer pH 8.0 containing 0.2 *M* NaCl and then lysed by sonication. After centrifugation at 27 000*g*, 0.1 m*M* PMSF was added and the supernatant was loaded onto a Q-Sepharose Fast Flow column (GE Healthcare). The proteins were eluted with a gradient of 200–500 m*M* NaCl in 20 m*M* Tris–HCl buffer pH 8.0. Flavoredoxin fractions were identified from the UV–Vis spectra. Ammonium sulfate (1.2 *M*) was added to the collected flavoredoxin fractions and insoluble materials were removed by centrifugation at 27 000*g*. The supernatant was then loaded onto a Hi-Trap Butyl Fast Flow column (GE Healthcare) and eluted with a gradient of 1.2–0.48 *M* ammonium sulfate in 50 m*M* phosphate buffer pH 7.0. The flavoredoxin fractions were loaded onto a HiLoad 16/60 Superdex column (200 prep grade; GE Healthcare) in 25 m*M* Tris–HCl pH 7.5. The apparent molecular weight of

Table 1
Data-collection and phasing statistics.

Values in parentheses are for the outer shell.

	Native	Methylmercuric chloride derivative
Beamline	SPring-8, BL41XU	SPring-8, BL44XU
Wavelength (Å)	0.7100	1.0000
Space group	$P3_121$	$P3_121$
Unit-cell parameters (Å)	$a = b = 53.35$, $c = 116.22$	$a = b = 53.5$, $c = 116.2$
Resolution (Å)	50.00–1.05 (1.09–1.05)	50.00–1.71 (1.77–1.71)
No. of observations	950955	205263
No. of unique reflections	89930 (8634)	21327 (2046)
Completeness (%)	99.6 (97.0)	99.6 (97.6)
$R_{\rm merge}$†	0.088 (0.573)	0.087 (0.158)
Multiplicity	10.6 (8.7)	9.6 (7.2)
$\langle I/\sigma(I)\rangle$	42.1 (3.7)	59.8 (14.0)
Phasing power	—	5.13
$R_{\rm Cullis\ ano}$‡	—	0.284
FOM (centric/acentric)	—	0.165/0.609

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation. ‡ $R_{\rm Cullis\ ano} = \langle \text{lack-of-closure}_{\rm ano}\rangle / \langle |F_{\rm PH}(+) - F_{\rm PH}(-)|\rangle$, where $F_{\rm PH}(+)$ and $F_{\rm PH}(-)$ are the amplitudes of the positive and negative counterparts of the Bijvoet pair.

37 000 Da calculated from the elution volume of the gel-filtration chromatography suggested that flavoredoxin forms a dimer in its native form (the calculated value from the amino-acid sequence is 20 800 Da). The purity of the flavoredoxin for the crystallization experiment was checked by SDS–PAGE.

2.2. Crystallization

The purified protein solution was concentrated to 25 mg ml^{-1} by centrifugation using a Vivaspin (5000 Da molecular-weight cutoff; Sartorius). Crystallization was carried out using the sitting-drop vapour-diffusion method. 2 µl flavoredoxin in buffer solution (25 m*M* Tris–HCl pH 7.5) and 2 µl reservoir solution were mixed on CrystalClear Strips plates (Hampton Research). Initial screening was performed using Crystal Screen Lite, PEG/Ion Screen and Grid Screen Ammonium Sulfate from Hampton Research as well as Wizard and Cryo screen kits from Emerald BioSystems. Crystals were obtained using Wizard I solution No. 7 containing 10%(*w*/*v*) PEG 8000, 0.2 *M* zinc acetate and 100 m*M* MES pH 6.0.

Heavy-atom derivative crystals were prepared by soaking native crystals overnight in a reservoir solution to which 1 m*M* methylmercuric chloride had been added.

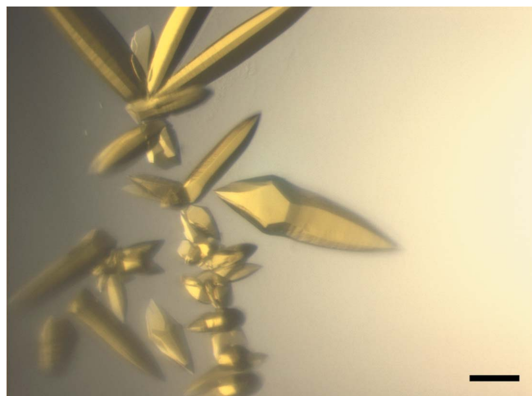

Figure 1
Crystals of flavoredoxin. The scale bar corresponds to 100 µm.

2.3. Data collection and crystallographic analysis

The native and methylmercuric chloride derivative crystals were soaked in mother liquor containing 30% glycerol as a cryoprotectant for a few seconds and then flash-cooled in a nitrogen stream at 100 K. Diffraction data were collected from the native crystal on the BL41XU beamline at SPring-8 (Hyogo, Japan) using an ADSC Q315 CCD detector system (camera distance 200.0 mm) and from the methylmercuric chloride derivative crystal on the BL44XU beamline at SPring-8 using a Bruker-AXS DIP6040 image-plate detector system (camera distance 300.0 mm). Diffraction data sets were indexed, integrated and scaled with the program *HKL*-2000 (Otwinowski & Minor, 1997). The diffraction experiments were performed at 100 K. The details of the diffraction experiments are summarized in Table 1.

3. Results

Flavoredoxin was successfully purified and formed crystals with typical dimensions of 0.1 × 0.1 × 0.3 mm in two weeks (Fig. 1). Diffraction data were collected to 1.0 Å resolution from the native crystal and to 1.7 Å resolution from the heavy-atom derivative crystal. The data statistics of the X-ray crystallographic experiments for both crystals are shown in Table 1. The crystals belonged to space group $P3_121$. The unit-cell parameters of the native and derivative crystals were almost identical (native, $a = b = 53.35$, $c = 116.22$ Å; methylmercuric chloride derivative, $a = b = 53.5$, $c = 116.2$ Å), suggesting that isomorphism was sufficiently preserved in both crystals. Assuming the presence of one flavoredoxin molecule in the asymmetric unit, the Matthews coefficient was estimated to be 2.29 Å^3 Da^{-1}, corresponding to a solvent content of 46%.

The single-wavelength anomalous dispersion (SAD) method was applied for the structure analysis of flavoredoxin. An anomalous difference Patterson map calculated with the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994) showed the presence of an anomalous scatterer (Fig. 2). The heavy-atom positions from the methylmercuric chloride derivative data were determined using *SnB* (Smith *et al.*, 1998). *SHARP* (de La Fortelle & Bricogne, 1997) was then used to refine the heavy-atom positions and occupancies and to calculate the initial phases, followed by density improvement with *SOLOMON* and *DM* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994; Fig. 3). The initial model was built automatically using *ARP*/*wARP* (Morris *et al.*,

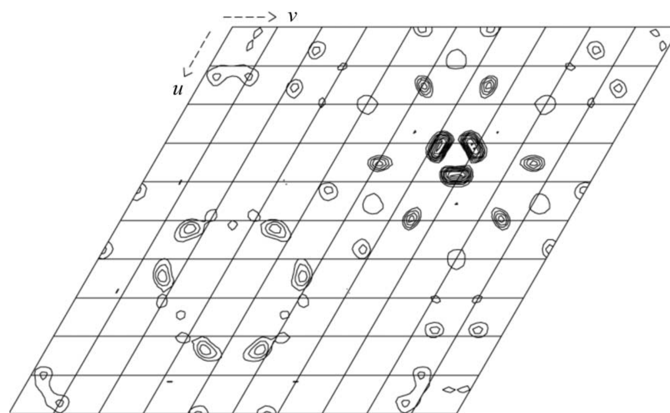

Figure 2
Harker section ($0 < u < 1$, $0 < v < 1$, $w = 0.33$) of the anomalous Patterson map for the methylmercuric chloride derivative at 1.71 Å resolution. Contours are drawn at the 1.0σ level starting at 1.0σ.

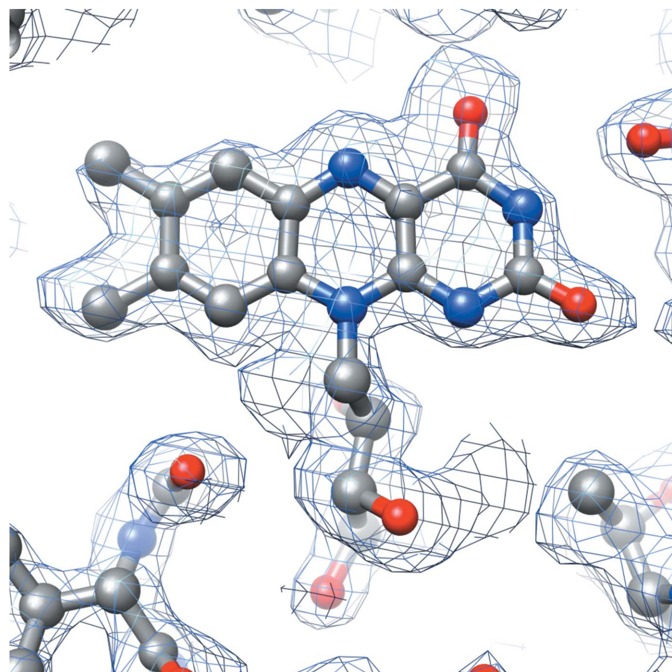

Figure 3
The experimental electron-density map after density improvement with *SOLOMON* and *DM* around the FMN-binding site, contoured at a level of 1.0σ. This figure was generated using *CHIMERA* (Pettersen *et al.*, 2004).

2003) and manually fitted for the unbuilt regions with *XFIT* (McRee, 1999). The structure has been solved (PDB code 2d5m) and a paper describing the refined structure in detail will be published elsewhere.

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