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Crystallization and preliminary X-ray crystallographic studies of the outer membrane cytochrome OmcA from *Shewanella oneidensis* MR-1

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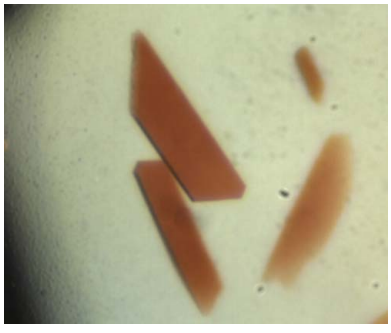
The outer membrane cytochrome OmcA functions as a terminal metal reductase in the dissimilatory metal-reducing bacterium *Shewanella oneidensis* MR-1. The ten-heme centers shuttle electrons from the transmembrane donor complex to extracellular electron acceptors. Here, the crystallization and preliminary crystallographic analysis of OmcA are reported. Crystals of OmcA were grown by the sitting-drop vapor-diffusion method using PEG 20 000 as a precipitant. The OmcA crystals belonged to space group $P2_1$, with unit-cell parameters $a = 93.0$, $b = 246.0$, $c = 136.6$ Å, $\alpha = 90$, $\beta = 97.8$, $\gamma = 90^\circ$. X-ray diffraction data were collected to a maximum resolution of 3.25 Å.

1. Introduction

Dissimilatory metal-reducing bacteria (DMRBs) such as *Shewanella oneidensis* MR-1 play an important role in the environmental cycling of Fe, Mn and other metals, the degradation of organic compounds and the reductive sequestration of radionuclide contaminants in sediments (Weber *et al.*, 2006). For example, *S. oneidensis* MR-1 has been shown to couple the oxidation of carbon sources (or molecular hydrogen, H_2) to the reduction of oxidized metals such as iron (Fe^{III}) and manganese (Mn^{IV}) (hydr)oxides (Myers & Nealson, 1988; Nealson & Saffarini, 1994). *S. oneidensis* MR-1 uses a set of multi-heme cytochromes (Weber *et al.*, 2006) to facilitate electron transfer from the cell to extracellular electron acceptors such as Fe^{III} oxides directly and/or indirectly *via* electron shuttles (Shi *et al.*, 2009). Specifically, the decaheme cytochromes encoded by the *mtrDEF-omcA-mtrCAB* gene cluster (Shi *et al.*, 2007; Coursolle & Gralnick, 2010) have been identified as the major electron-carrier proteins utilized by *S. oneidensis* MR-1 to transfer electrons across the outer membrane (OM). MtrA, MtrB and MtrC form a transmembrane complex (MtrCAB) that has been shown to conduct electrons across the bacterial OM (Hartshorne *et al.*, 2009). MtrF, MtrD and MtrE can form a similar trans-OM electron-transport complex (MtrFDE) that is homologous to MtrCAB (Bücking *et al.*, 2010; Coursolle & Gralnick, 2010). A recent crystal structure of MtrF has provided important molecular details of how extracellular electron transfer might occur (Clarke *et al.*, 2011). OmcA, a homolog of MtrC and MtrF, is a decaheme *c*-type cytochrome with a molecular mass of 85 kDa located on the cell surface of *S. oneidensis* MR-1 that can serve as a terminal reductase to transfer electrons to an insoluble mineral substrate at the microbe–mineral interface (Shi *et al.*, 2006). As the molecular structure of OmcA has not been determined, a thorough understanding of the mechanistic basis of dissimilatory metal reduction involving OmcA remains lacking. Here, we report the crystallization and preliminary X-ray diffraction analysis of OmcA at a resolution of 3.25 Å.



2. Materials and methods

2.1. Protein expression and purification

Multiheme cytochromes are difficult to obtain using classic *Escherichia coli* expression systems because specific maturation proteins are required for the effective incorporation of multiple heme moieties into expressed proteins. Thus, the expression of OmcA was

performed in *S. oneidensis* MR-1 (Shi *et al.*, 2005) using a construct lacking a single N-terminal cysteine residue that accommodates a post-translational lipid modification (Myers & Myers, 2004). It has been shown that the redox properties and metal reductase activities are not altered by deletion of the lipid-binding site (Eggleston *et al.*, 2008). The cell-culture conditions and expression of OmcA were the same as previously described for MtrA (Shi *et al.*, 2005). To isolate OmcA, the cell pellets were resuspended in ice-cold buffer *A* [20 m*M* Na HEPES pH 7.8, 5 m*M* β-mercaptoethanol, 150 m*M* NaCl, 0.05%(*w*/*v*) CHAPS], to which protease inhibitor (Complete, Roche Diagnostic, Indianapolis, Indiana, USA) was added following the manufacturer's instructions. The cells were lysed by passage through a French press three times at 55 MPa. Unbroken cells and debris were removed by centrifugation at 15 000*g* and 277 K for 30 min. The supernatant was transferred into an ultracentrifugation tube and centrifuged at 150 000*g* for 1 h. The supernatant was loaded onto a 1 × 5 cm column of Ni^{2+}–NTA Sepharose (GE Healthcare, Piscataway, New Jersey, USA) pre-equilibrated with buffer *A*. The column was washed with 25 ml of the following ice-cold buffers in sequential order: buffer *B* (buffer *A* + 10% glycerol), buffer *C* (buffer *B* + 10 m*M* imidazole) and buffer *D* (buffer *B* + 40 m*M* imidazole). Finally, it was eluted with 10 ml buffer *E* (buffer *B* + 250 m*M* imidazole).

The identity of OmcA was confirmed by Western blot analysis with anti-V5 antibody (Invitrogen, Carlsbad, California, USA). The fractions containing OmcA were pooled and concentrated using Amicon Ultra centrifugal devices (Millipore, Billerica, Massachusetts, USA). The concentrated OmcA was loaded onto a HiLoad 16/60 Superdex 200 column and was eluted with buffer *F* [20 m*M* Tris–HCl pH 7.8, 150 m*M* NaCl] using an ÄKTAexplorer FPLC system (GE Healthcare, Piscataway, New Jersey, USA). The homogeneity of the purified OmcA (approximately 79.2 kDa, consisting of 740 amino-acid residues) was confirmed by staining with GelCode stain reagent (Pierce, Rockford, Illinois, USA) following SDS–PAGE. Small-angle X-ray scattering showed that OmcA forms a monomer in solution and undergoes redox-state-dependent conformational change (Johs *et al.*, 2010). The extinction coefficient of oxidized OmcA was determined to be 916 mM^{-1} cm^{-1} by relating the absorption at 410 nm to a concentration series determined using a Bradford assay. All protein concentrations were determined by measuring the absorption at 410 nm.

2.2. Crystallization

Following purification, OmcA was dialyzed in a buffer solution consisting of 20 m*M* Tris–HCl pH 7.8, 150 m*M* NaCl, 0.1% CHAPS, 5% glycerol and the protein was concentrated to 10 mg ml^{-1} for crystallization trials. Initial OmcA crystallization conditions were obtained from high-throughput microbatch-under-oil crystallization screening at the Hauptman–Woodward Medical Research Institute, Buffalo, New York, USA (Luft *et al.*, 2003). The microbatch crystallization setup contained 0.2 µl protein solution (10 mg ml^{-1}) mixed with 0.2 µl crystallization-cocktail solution [20%(*v*/*v*) polyethylene glycol 20 000, 0.1 *M* Tris–HCl pH 8.0, 0.1 *M* $MgCl_2$] and covered with paraffin oil (EMD Chemicals Inc., Gibbstown, New Jersey, USA) at 295 K. Small red-colored crystals with a plate-like morphology were observed after three weeks. During crystallization optimization of the microbatch setups grown under oil, crystal manipulation for cryogenic preservation proved to be difficult owing to the small size of the OmcA crystals and the highly viscous nature of the mother liquor. As a result, exhaustive efforts were thereafter focused on growing crystals at an overall lower supersaturation using the hanging-drop vapor-diffusion method. The purified protein was dialyzed into a low ionic strength buffer (20 m*M* Tris–HCl pH 7.8) and concentrated to 8 mg ml^{-1} prior to hanging-drop vapor-diffusion crystallization. Diffraction-quality crystals of OmcA were obtained after 3–4 weeks from a hanging-drop gradient expansion with a reservoir solution consisting of 8–11%(*v*/*v*) polyethylene glycol 20 000, 0.1 *M* Tris–HCl pH 7.8, 0.1 *M* $MgCl_2$ at 295 K. In the setup, a hanging drop consisting of 2 µl protein solution mixed with 2 µl reservoir solution was equilibrated over a total reservoir volume of 500 µl. OmcA crystals grown using the hanging-drop vapor-diffusion method are shown in Fig. 1.

2.3. X-ray diffraction data collection and processing

Prior to X-ray diffraction data collection, a crystal of OmcA was momentarily placed in reservoir solution containing a cryoprotectant [25% glycerol, 11%(*v*/*v*) polyethylene glycol 20 000, 0.1 *M* Tris–HCl pH 7.8, 0.1 *M* $MgCl_2$] at 277 K and flash-cooled in liquid nitrogen. X-ray diffraction data to 3.25 Å resolution were collected from a single OmcA crystal at 100 K over a range of 190° (crystal-to-detector distance 450 mm, 1.0° oscillation, 60 s exposure) using an ADSC Quantum 315 CCD detector on the BioCARS 14-BMC beamline (λ = 0.979 Å) at the Advanced Photon Source (Chicago, Illinois, USA). The data were integrated using *XDS* (Kabsch, 2010); *SCALA* (Winn *et al.*, 2011) was used for scaling and merging during data analysis.

3. Results and discussion

The outer membrane decaheme *c*-type cytochrome OmcA from *S. oneidensis* MR-1 was expressed and purified to homogeneity under aerobic conditions. The isolated OmcA was crystallized using the hanging-drop vapor-diffusion method. During initial X-ray diffraction screening, many crystals showed twinning and elliptical diffraction spots as OmcA crystals have a propensity to grow as thin stacked plates. Owing to the very small size of the crystals used in this study, crystal decay resulting from radiation damage further complicated data collection. Precooling the crystallization drops to 277 K and variation of the cryoprotectant composition and the time of soaking significantly improved the diffraction quality. The best X-ray diffraction data set was collected from a single crystal at 100 K. The data

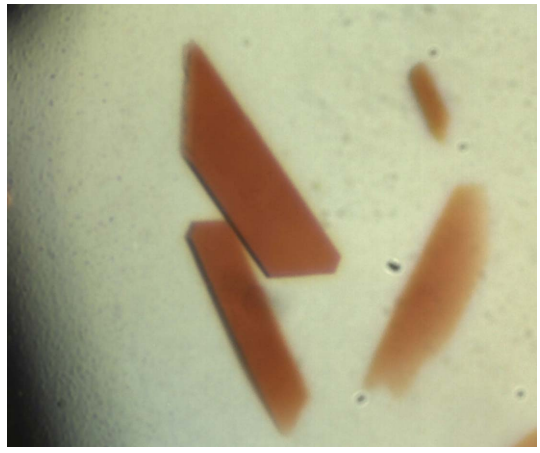


Figure 1
OmcA crystals grown using the hanging-drop vapor-diffusion method at 295 K with a reservoir solution consisting of 10%(*v*/*v*) polyethylene glycol 20 000, 0.1 *M* Tris–HCl pH 7.8, 0.1 *M* $MgCl_2$. The crystals grew with a plate-like morphology; the longest dimension was measured as approximately 0.15–0.2 mm. All OmcA crystals were clearly red in color; however, the equipment used to obtain the image could not be accurately calibrated to capture this true red color.

Table 1
X-ray diffraction data collection.

Values in parentheses are for the highest resolution shell.

X-ray source	APS BioCARS 14-BMC
Data-collection temperature (K)	100
Wavelength (Å)	0.979
No. of images	190
Oscillation range (°)	1
Exposure time (s)	60
Unit-cell parameters (Å)	a = 93.0, b = 246.0, c = 136.6, α = 90, β = 97.8, γ = 90
Asymmetric unit contents	8 molecules
Solvent content (%)	49.4
Space group	$P2_1$
No. of reflections	364673
No. of unique reflections	94591 (13632)
Resolution range (Å)	47.6–3.25 (3.43–3.25)
Multiplicity	3.9 (3.7)
$\langle I/\sigma(I)\rangle$	12.2 (4.8)
$R_{\rm merge}$† (%)	11.3 (28.7)
$R_{\rm p.i.m.}$ (%)	6.6 (17.2)
Data completeness (%)	99.2 (97.9)
Wilson plot B factor (Å^2)	43.3

† $R_{\rm merge} = 100 \times \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the mean value of i measurements of the reflection.

were processed to 3.25 Å resolution with a completeness of 99.2% and an overall $R_{\rm p.i.m.}$ of 6.6% (see Table 1). The OmcA crystal belonged to space group $P2_1$, with unit-cell parameters a = 93.0, b = 246.0, c = 136.6 Å, α = 90, β = 97.8, γ = 90°. *phenix.xtriage* (Zwart *et al.*, 2005) from the *PHENIX* suite (Adams *et al.*, 2002) was also used to verify that no twinning could be detected in the reported data set. Calculation of the Matthews coefficient ($V_{\rm M}$ = 2.43 Å^3 Da^{-1}; molecular weight = 79.2 kDa) indicated the presence of eight molecules per asymmetric unit (Matthews, 1968). A summary of all X-ray crystallographic data-collection statistics is given in Table 1.

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