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Crystallization and preliminary X-ray diffraction studies of the hyperthermophilic archaeal sulredoxin having the unique Rieske [2Fe–2S] cluster environment

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The hyperthermophilic archaeal sulredoxin from *Sulfolobus tokodaii* is a water-soluble high-potential Rieske [2Fe–2S] protein with unique pH-dependent redox properties compared with its mesophilic homologues in cytochrome bc_1/b_6f complexes. The oxidized recombinant sulredoxin has been crystallized by the hanging-drop vapour-diffusion method using 30%(*v*/*v*) polyethylene glycol 400, 0.1 *M* cadmium chloride and 0.1 *M* sodium acetate pH 4.6. The crystals diffracted to beyond 2.0 Å resolution and belong to the cubic space group $F4_132$, with unit-cell parameter $a = 163.00 \pm 0.05$ Å. The asymmetric unit contains one sulredoxin molecule. Three-wavelength MAD data were collected.

1. Introduction

Proteins containing Rieske-type [2Fe–2S] clusters play important electron-transfer roles in various key pathways such as aerobic respiration, photosynthesis and the biodegradation of some alkene and aromatic compounds (Berry *et al.*, 2000; Link, 1999; Mason & Cammack, 1992; Trumpower & Gennis, 1994). In contrast to regular plant/vertebrate-type [2Fe–2S] ferredoxins, which have complete cysteinyl ligations, the Rieske-type cluster has an asymmetric iron–sulfur core with the S^{γ} atom of each of the two cysteine residues coordinated to one Fe site and the N^{δ} atom of each of the two histidine residues coordinated to the other Fe site.

Two different types of Rieske clusters have been observed in proteins. One type displays higher E_m values of approximately +150 to +490 mV and occurs in proton-translocating cytochrome bc_1/b_6f complexes, being involved in not only electron transfer but also in substrate binding and oxidation at the quinol-oxidizing Q_0 site (Berry *et al.*, 2000; Brugna *et al.*, 1999; Iwasaki, Matsuura *et al.*, 1995; Iwata *et al.*, 1998; Link, 1999; Samoilova *et al.*, 2002; Trumpower & Gennis, 1994; Zhang *et al.*, 1998; Zu *et al.*, 2003). The other type displays lower E_m values of approximately −150 to −50 mV at least up to pH 10 and has been found in a diverse group of archaeal and bacterial multi-component terminal oxygenases and soluble Rieske-type ferredoxins (Cosper *et al.*, 2002; Couture *et al.*, 2001; Dikanov *et al.*, 2004; Kounosu *et al.*, 2004; Link, 1999; Link *et al.*, 1996; Mason & Cammack, 1992; Zu *et al.*, 2003). X-ray crystal structural analyses of several Rieske-type protein domains, including examples from each of these classes (Bönisch *et al.*, 2002; Carrell *et al.*, 1997; Colbert *et al.*, 2000; Hunsicker-Wang *et al.*, 2003; Iwata *et al.*, 1996; Kauppi *et al.*, 1998), show heightened structural variations of the immediate cluster environment, which have tentatively been invoked to explain the marked variations of the pH-dependent E_m of the clusters (Colbert *et al.*, 2000; Hunsicker-Wang *et al.*, 2003; Link, 1999). However, because of the low amino-acid sequence homology between these proteins, further structural characterization of a stable Rieske protein in different oxidation/pH states, in conjunction with site-directed mutagenesis studies, is required to establish the critical structural elements in the modulation of the biological electron- and proton-transfer functions of this protein family.

Belonging to the high-potential class of the Rieske protein family (typical MW = 20–25 kDa), the protein of interest is the hyperthermostable sulredoxin from the hyperthermoacidophilic archaeon *Sulfolobus tokodaii* strain 7 ($E_{m,\ acid\ pH}$ of ∼+190 mV), which is weakly homologous to the extrinsic cluster-binding domain of cytochrome bc_1-associated high-potential Rieske proteins with a solvent-exposed consensus disulfide linkage (DDBJ accession No. AB023295; Iwasaki, Isogai *et al.*, 1995; Iwasaki *et al.*, 1996, 2004; Kounosu *et al.*, 2004). This archaeal 12 kDa protein shows some unusual sequence features such as (i) the inherent absence of the transmembrane domain and (ii) a lack of the conserved hydroxyl groups near the cluster that modulate the cluster E_m in cytochrome bc_1 complexes (Denke *et al.*, 1998; Guergova-Kuras *et al.*, 2000; Schröter *et al.*, 1998). The latter observation suggests a unique hydrogen-bonding pattern in the immediate cluster environment of sulredoxin. In accordance with this, the pH-dependent E_m and spectral transitions of sulredoxin (Iwasaki *et al.*, 1996, 2004) are

substantially different from those of other cytochrome *bc*-associated high-potential Rieske proteins (Link *et al.*, 1992). Recombinant sulredoxin has been overproduced in *Escherichia coli* and can be obtained in appropriate forms for isotope labelling, site-directed mutagenesis and various spectroscopic studies (Dikanov *et al.*, 2004; Iwasaki *et al.*, 2004; Kounosu *et al.*, 2004; Iwasaki *et al.*, manuscript in preparation). Here, we present the crystallization in the oxidized conformation near the acidic edge of the pH-dependent spectral transitions (*i.e.* the fully protonated state) of *S. tokodaii* sulredoxin in a form suitable for high-resolution X-ray studies and preliminary X-ray data analysis.

2. Methods and results

2.1. Protein preparation

The *sdx* gene coding for the high-potential archaeal sulredoxin (DDBJ accession No. AB023295) of *S. tokodaii* strain 7 (JCM 10545^{T}; formerly *Sulfolobus* sp. strain 7; Suzuki *et al.*, 2002) has been cloned, sequenced and heterologously overexpressed in *Escherichia coli* BL21-CodonPlus(DE3)-RIL cells (Stratagene) harbouring plasmid pET28a (Novagen) as described previously (Iwasaki *et al.*, 2004; Kounosu *et al.*, 2004). The recombinant holo-sulredoxin with a hexahistidine tag at the N-terminus was purified as reported in detail elsewhere (Iwasaki *et al.*, 2004; Kounosu *et al.*, 2004). After proteolytic removal of the hexahistidine tag from the recombinant protein for 16–22 h at 297 K using a Thrombin Cleavage Capture Kit (Novagen) according to the manufacturer's instructions, the sample was fully reoxidized with a small amount of potassium ferricyanide for a few minutes, which was subsequently removed by gel-filtration chromatography (Sephadex G-50; Amersham Pharmacia Biotech) eluted with 20 m*M* Tris–HCl, 350 m*M* NaCl pH 7.5. The resultant sample was concentrated with a Centriprep-10 apparatus (Amicon) to ∼0.3–0.5 m*M* and stored frozen (193 K) until use. The purity of the sample was assessed by SDS–PAGE. Its N-terminal amino-acid sequence, the pH-dependent visible circular-dichroism transitions in the oxidized and reduced states at 293 K and the electron paramagnetic resonance properties of the dithionite-reduced protein at 10 K ($g_{z,y,x}$ = 2.008, 1.91, 1.79; Dikanov *et al.*, 2004; Iwasaki, Isogai *et al.*, 1995; Iwasaki *et al.*, 2004; Kounosu *et al.*, 2004) were verified with a Perkin–Elmer Applied Biosystems Procise Model 490 gas-phase sequencer, a JASCO J720 spectropolarimeter and a JEOL JEX-RE3X spectrometer equipped with an ES-CT470 Heli-Tran cryostat system and a Scientific Instruments digital temperature indicator/controller Model 9650, respectively (data not shown).

Table 1
Data-processing statistics.

Values in parentheses are for the outer shell.

		MAD		
	Single wavelength	Peak	Edge	Remote
Wavelength (Å)	1.6400	1.7386	1.7414	1.6400
Space group	$F4_132$			
Unit-cell parameter (Å)	162.88	163.05		
Resolution range (Å)	60.00–2.00 (2.07–2.00)	57.71–2.20 (2.28–2.20)	57.61–2.20 (2.28–2.20)	57.69–2.20 (2.28–2.20)
No. measured reflections	106448	288608	287873	295068
No. unique reflections	22478	18361	17629	18638
Redundancy	4.74	15.72	16.33	16.06
Completeness (%)	98.7 (89.6)	100.0 (100.0)	100.0 (100.0)	100.0 (100.0)
$R_{\rm merge}$† (%)	7.5 (25.8)	8.9 (28.4)	8.8 (29.3)	8.2 (20.4)
$I/\sigma(I)$	16.6 (3.0)	20.9 (8.2)	23.8 (9.9)	24.3 (12.7)
Mosaicity (°)	0.358	0.373	0.387	0.387

† $R_{\rm merge} = \sum_{hkl}\sum_j |I_j(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_j \langle I(hkl)\rangle$, where $I_j(hkl)$ and $\langle I(hkl)\rangle$ are the intensity of measurement j and the mean intensity for the reflection with indices hkl, respectively.

2.2. Crystallization

Preliminary screening was carried out by standard hanging-drop vapour diffusion in Linbro plates at 277–293 K with 0.5 ml reservoirs of commercially available sparse-matrix screening kits (Hampton Research Crystal Screen kits I and II and Emerald BioStructures kits Cryo I and II). Several different crystal forms were obtained. Optimized crystals were obtained under aerobic conditions in 2–3 d at 293 K by combining 0.5–10 µl protein solution with 0.5–1.5 µl reservoir solution containing 30%(*v*/*v*) polyethylene glycol 400, 0.1 *M* cadmium chloride and 0.1 *M* sodium acetate pH 4.6. The crystals grew to maximum dimensions of 0.2 × 0.2 × 0.2 mm (Fig. 1). The crystals were transferred into a cryoprotective solution containing 10%(*v*/*v*) glycerol in the same reservoir solution for flash-cooling in liquid nitrogen.

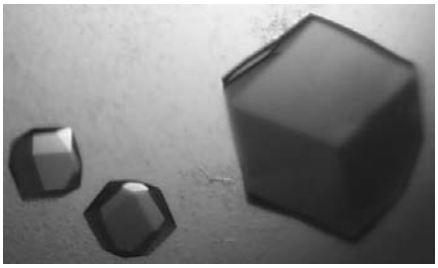

Figure 1
Typical crystals of *S. tokodaii* sulredoxin. The maximum dimensions of the cubic crystals (right) are approximately 0.2 × 0.2 × 0.2 mm.

2.3. Crystallographic data collection and processing

X-ray diffraction data of the archaeal sulredoxin were collected from flash-frozen crystals using a Rigaku/MSC Jupiter 210 CCD detector installed on the BL26B2 beamline at SPring-8, Japan. Diffraction data were recorded at a distance of 200.00 mm, with a total oscillation range of 135° for MAD and 54° for single-wavelength data collection, respectively, with a step size of 1.0° for an exposure time of 20 s. Of the several different crystal forms obtained in the initial screening, the cubic sulredoxin crystals (Fig. 1) were found to diffract to a resolution beyond 2.0 Å and to belong to space group $F4_132$, with unit-cell parameter $a = 163.00 \pm 0.05$ Å (Table 1). Assuming one sulredoxin molecule per asymmetric unit, the Matthews coefficient is 3.4 Å^3 Da^{-1}, corresponding to a solvent content of 65% (Matthews, 1968).

Because of the low amino-acid sequence similarity between archaeal sulredoxin (DDBJ accession No. AB023295; Kounosu *et al.*, 2004) and other Rieske-type [2Fe–2S] proteins of known crystal structure (*e.g.* PDB code 1rie, Iwata *et al.*, 1996; PDB code 1rfs, Carrell *et al.*, 1997; PDB code 1fqt, Colbert *et al.*, 2000), our initial molecular-replacement trials for the phase determination were unsuccessful. Therefore, a three-wavelength MAD experiment (Hendrickson & Ogata, 1996) using the anomalous scattering of the intrinsic Fe atoms was conducted in conjunction with X-ray fluorescence spectra recorded in the vicinity of the Fe *K* absorption edge of the flash-frozen sulredoxin crystals at 100 K. The MAD data were collected at 100 K at 1.7414 Å (the inflection point of the X-ray fluorescence spectrum, f' minimum), 1.7386 Å (the maximum of the fluorescence spectrum, f'' maximum) and 1.6400 Å (the remote high-

energy wavelength) and were processed with the *d*TREK* data-processing package (Pflugrath, 1999) from the *CrystalClear* suite (Rigaku/MSC) (Table 1). The experimental MAD phases were calculated in the 60–2.2 Å resolution range with the program *SOLVE* (Terwilliger & Berendzen, 1999), which automatically identified the approximate position of a Rieske-type [2Fe–2S] cluster core. Density modification with the program *DM* (Cowtan, 1994) using solvent flattening and histogram matching significantly improved the phases and resulted in a traceable electron-density map. Construction, revision and analysis of atomic models using the *S. tokodaii* sulredoxin sequence are currently in progress.

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