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Crystallization and preliminary crystallographic analysis of Rieske iron–sulfur protein II (soxF) from *Sulfolobus acidocaldarius*

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An archaeal Rieske iron–sulfur protein has been crystallized for the first time. The genetically constructed soluble form of the soxF protein was expressed in *E. coli*. It contains a correctly inserted [2Fe–2S] cluster. The authentic soxF protein is part of a terminal oxidase complex in the respiratory chain of the hyperthermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* (DSM 639). The enzyme crystallizes in the space group $P6_1$ or $P6_5$, with unit-cell parameters $a = b = 80.19$, $c = 75.69$ Å. A complete data set has been collected to 1.64 Å resolution at 100 K.

1. Introduction

The two Rieske iron–sulfur proteins of *S. acidocaldarius* (German Collection of Microorganisms and Cell Cultures, DSM 639) and the protein from *Pyrobaculum aerophilum* (Henninger *et al.*, 1999) are the only archaeal members of this protein family so far characterized in detail. In contrast to the Rieske proteins from prokarya and eukarya, which are known to be essential subunits of the cytochrome bc_1/b_6f complexes, the soxF protein has a novel structure clearly different from these complexes. It is a constituent of a terminal oxidase complex (soxM; Lübben *et al.*, 1994; Castresana *et al.*, 1995) together with a cytochrome *b* homologue, suggesting a structure with a suggested function similar to a bc_1 complex.

The soxF protein was the first member of the family of the membrane-associated Rieske proteins to be heterologously expressed in *Escherichia coli* with a correctly inserted iron–sulfur cluster (Schmidt *et al.*, 1997).

Here, we report the crystallization and preliminary crystallographic analysis of a soluble form of this protein created by deletion of the 45 N-terminal amino acids. The X-ray structure will be used to investigate the structure and function of respiratory Rieske proteins and to test predictions based on the known structures from eukarya (Iwata *et al.*, 1996; Carrell *et al.*, 1997) by site-directed mutagenesis. Owing to the extreme growth conditions of *Sulfolobus* (353 K, pH 3), it will be of special interest to compare the structure of the *Sulfolobus* Rieske II protein with these structures. Based on the low sequence homology (around 12 and 18% sequence identity, respectively, with the known structures from bovine heart mitochondria and spinach chloroplasts) and the occurrence of a sequence insertion in the cluster-binding domain, a deviating structure of the [2Fe–2S] cluster and/or the cluster-binding domain in this archaeal protein is expected.

2. Materials and methods

2.1. Crystallization

The Rieske iron–sulfur protein II (soxF) from *S. acidocaldarius* was heterologously expressed in *E. coli* and purified according to Schmidt *et al.* (1997). For crystallization experiments, a solution of the protein in 0.2 m*M* EDTA, 10 m*M* Tris–HCl pH 7.5 with a protein concentration of 20 mg ml^{-1} was used.

First crystallization trials using sparse-matrix screening including Hampton Crystal Screens I and II (Jancarik & Kim, 1991; Cudney *et al.*, 1994) showed that the protein crystallizes under a variety of different crystallization conditions, forming crystals of hexagonal shape. However, these crystals were not suitable for X-ray measurements owing to small size or growth defects. By optimization of crystallization parameters, the quality of the crystals was improved significantly. The best crystals were obtained with 10% polyethylene glycol 8000, 0.2 *M* magnesium acetate and 0.1 *M* sodium cacodylate pH 7.0 in sitting-drop vapour-diffusion experiments at 293 K using Cryschem Plates (Hampton Research, USA). With 500 µl reservoir solution and drops consisting of 2 µl protein solution and 2 µl reservoir solution, crystals appeared after two weeks and grew to maximum dimensions of around 0.4 × 0.4 × 0.5 mm in four weeks (Fig. 1).

2.2. X-ray data collection and processing

Prior to X-ray data collection, the crystals were transferred to a cryoprotectant buffer

consisting of 40% glycerol, 10% polyethylene glycol 8000, 0.2 *M* magnesium acetate and 0.1 *M* sodium cacodylate pH 7.5. The crystals were picked up with a nylon loop and flash-cooled at 100 K in a stream of gaseous nitrogen from a Cryostream cooling system (Oxford Cryosystems, Oxford, England). X-ray diffraction data were collected at a wavelength of 1.5418 Å using a Siemens rotating-anode X-ray generator operated at 45 kV and 100 mA (Cu $K\alpha$ radiation, graphite monochromator, 0.3 mm collimator) and a 30 cm MAR image-plate detector (MAR Research, Germany).

The crystal-to-detector distance was set to 100.0 mm (1.64 Å maximum resolution). 180 rotation images were collected with a rotation angle of 1° and an exposure time of 300 s. Using *DENZO* for data reduction and *SCALEPACK* (Otwinowski & Minor, 1997) for scaling, the raw data were processed to a resolution of 1.64 Å.

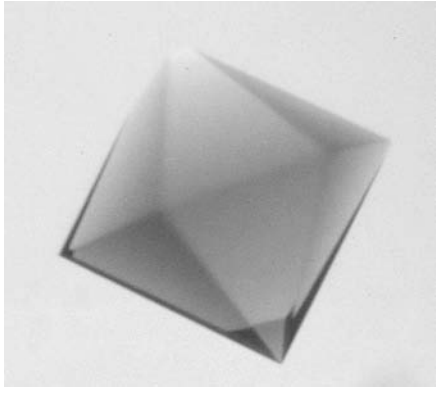

Figure 1
A typical hexagonal crystal of Rieske iron–sulfur protein II from *S. acidocaldarius* grown in 10% polyethylene glycol, 0.2 *M* magnesium acetate and 0.1 *M* sodium cacodylate pH 7.0. The dimensions of the crystal are approximately 0.4 × 0.4 × 0.2 mm.

3. Results and discussion

A complete data set has been collected to a resolution of 1.64 Å using a single crystal of dimensions 0.4 × 0.4 × 0.3 mm. Detailed data-collection statistics are given in Table 1. Using results from autoindexing by *DENZO* and pseudo-precession photographs created by the program *PATTERN* (Lu, 1998), the space group was determined to be either $P6_1$ or $P6_5$, with unit-cell parameters $a = b = 80.19$, $c = 75.69$ Å, $\alpha = \beta = 90$, $\gamma = 120°$. From the calculation of the Matthews coefficient (Matthews, 1968) $V_m = 3.20$ Å^3 Da^{-1}, it can be concluded that the asymmetric unit contains one Rieske protein molecule (the molecular weight of the truncated protein is 21 860 Da), corresponding to a relatively high solvent content of 62%.

Owing to the low sequence homology and structural differences expected from sequence alignments, attempting to solve the structure by molecular-replacement using known structures of eukaryotic Rieske proteins would not seem promising. Instead, it is intended to solve the structure by a combination of multiple isomorphous replacement (MIR) and multiple-wavelength anomalous dispersion (MAD), taking advantage of the iron–sulfur cluster of the protein. The search for heavy-atom derivatives is in progress.

The solution of the structure is expected not only to reveal the exact geometry of the iron–sulfur cluster and its ligands, but also to provide clues to the structural basis for the acidity tolerance and the thermotolerance of the protein.

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Table 1
X-ray data-collection statistics.

Values for the resolution range 1.68–1.64 Å are given in parentheses.

Number of crystals	1
Crystal dimensions (mm)	0.4 × 0.4 × 0.3
Temperature (K)	100
Resolution range (Å)	30–1.64
Space group	$P6_1$ or $P6_5$
Unit-cell parameters (Å)	80.19, 80.19, 75.69
Number of observations	651160
Number of unique reflections	33926
Completeness (%)	100.0 (100.0)
Average redundancy	10.7
Mosaicity (°)	0.4
Average $I/\sigma(I)$	15.4
$I > 3\sigma(I)$ (%)	89.6 (69.1)
$R_{\rm sym}$ (%)	5.3 (29.7)

† $R_{\rm sym}(I) = \sum_{hkl}\sum_i I_i(hkl) - \overline{I}(hkl)/\sum_{hkl}\sum_i I_i(hkl)$.

References

Carrell, C. J., Zhang, H., Cramer, W. A. & Smith, J. L. (1997). *Structure*, **5**, 1613–1625.
Castresana, J., Lübben, M. & Saraste, M. (1995). *J. Mol. Biol.* **250**, 202–210.
Cudney, R., Patel, S., Weisgraber, K., Newhouse, Y. & McPherson, A. (1994). *Acta Cryst.* D**50**, 414–423.
Henninger, T., Anemüller, S., Fitz-Gibbon, S., Miller, J. H., Schäfer, G. & Schmidt, C. L. (1999). *J. Bioenerg. Biomembr.* **31**, 119–128.
Iwata, S., Saynovits, M., Link, T. A. & Michel, H. (1996). *Structure*, **4**, 567–579.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl. Cryst.* **24**, 409–411.
Lu, G. (1998). *J. Appl. Cryst.* **32**, 375–376.
Lübben, M., Arnaud, S., Castresana, J., Warne, A., Albracht, S. P. J. & Saraste, M. (1994). *Eur. J. Biochem.* **224**, 151–159.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Schmidt, C. L., Hatzfeld, O. M., Petersen, A., Link, T. A. & Schäfer, G. (1997). *Biochem. Biophys. Res. Commun.* **234**, 283–287.