Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction analysis of ferredoxin-NADP(H) reductase from *Rhodobacter capsulatus*

Inmaculada Pérez-Dorado,[a] Cristian Bittel,[b] Néstor Cortez[b] and Juan A. Hermoso[a]*

[a]Grupo de Cristalografía Macromolecular y Biología Estructural, Instituto Rocasolano, CSIC, Serrano 119, 28006 Madrid, Spain, and [b]Instituto de Biología Molecular y Celular de Rosario (IBR), Facultad de Ciencias Bioquímicas y Farmaceúticas, Universidad Nacional de Rosario, Argentina

Correspondence e-mail: xjuan@iqfr.csic.es

Received 22 July 2004
Accepted 18 October 2004

Ferredoxin-NADP(H) reductase (272 amino acids) from *Rhodobacter capsulatus* (FPR) has recently been postulated to be involved in the antioxidant response and to facilitate the provision of reduced flavodoxin for the reduction of nitrogenase. Crystallization trials of recombinant FPR were carried out at 291 K by the hanging-drop vapour-diffusion method. Orthorhombic crystals (unit-cell parameters a = 69.3, b = 93.6, c = 103.5 Å) were obtained. However, their diffraction pattern was not satisfactory and an extensive detergent screening was carried out over the initial crystallization conditions. The introduction of n-heptyl-β-D-thioglucoside produced new trigonal crystals (space group $P3_121$; unit-cell parameters a = b = 120.5, c = 51.1 Å) that diffracted to 1.8 Å resolution at beamline BM16 at the ESRF. Preliminary structural analysis indicated that detergent molecules could increase the quality of diffraction of FPR crystals by stabilizing the disordered regions of the protein and by increasing the number of contacts in the crystal packing.

1. Introduction

Ferredoxin-NADP(H) reductase (FPR) from the phototrophic bacterium *Rhodobacter capsulatus* is a member of the ferredoxin-NADP(H) reductase family (FNR; EC 1.18.1.2). It consists of 272 amino acids (MW = 30 138 Da) and possesses a non-covalently bound FAD molecule as a prosthetic group (Bittel *et al.*, 2003). FNR enzymes are capable of the transfer of two electrons between NADP(H) and obligatory one-electron carriers (ferredoxin or flavodoxin) through a reversible mechanism. This ability is a consequence of the electronic structure of the isoalloxazine group of FAD, which may adopt three possible redox states: oxidized (quinone), one-electron reduced (semiquinone) and reduced (hydroquinone) (Carrillo & Ceccarelli, 2003).

FNRs are folded into two domains: the N-terminal domain, which carries the FAD molecule, and the C-terminal domain, which has a nucleotide-binding fold that is capable of binding $NADP^+$/NADPH (Arakaki *et al.*, 2000). The differences in the cofactor conformations allow differentiation between a plastidic class of FNR present in plastids and cyanobacteria that contain an extended FAD molecule and a bacterial class of FNR which has a twisted dinucleotide (Ceccarelli *et al.*, 2004).

Bacterial class reductases exhibit significantly low k_{cat} values when compared with plastidic reductases (Bittel *et al.*, 2003; Ceccarelli *et al.*, 2004). The lower catalytic efficiency of bacterial FNRs might evolve from the slow electron-flow processes that these enzymes are a part of. FPR from *R. capsulatus* showed NifF (the flavodoxin encoded by nitrogen fixation, *nif*, genes) turnover values compatible with electron currents through the nitrogenase pathway: this supports its possible role in the nitrogen-fixation process (Bittel *et al.*, 2003).

R. capsulatus is a facultative phototropic microorganism that can adapt its metabolism to aerobic or anaerobic environments. Although FPR levels do not change with aeration conditions, it has been observed that oxidative-stress conditions enhanced its expression by a presently unknown mechanism. This suggests that FPR may play a role in the antioxidant protective system of the cell (Bittel *et al.*, 2003).

In this work, we have obtained FPR crystals in two different forms in the absence and presence of a detergent, n-heptyl-β-D-thioglucoside, which has proved to be essential in stabilizing the disordered regions of the crystal.

2. Experimental

2.1. Expression and purification

Recombinant FPR was expressed as a C-terminal fusion of a Trx-tag thioredoxin protein using the pET-32a vector (Novagen) in BL21(DE3)pLys *Escherichia coli* cells as previously described (Bittel *et al.*, 2003). After isolation by affinity chromatography on an Ni–nitriloacetic acid affinity column, the fusion

flavoprotein was eluted with 300 m*M* imidazole, digested with enterokinase and purified as specified in Bittel *et al.* (2003). The concentrations of protein samples were measured spectrophotometrically using the FAD coefficient absorption at 452 nm (ε_{452} = 12.5 mM^{-1} cm^{-1}).

2.2. Crystallization

Preliminary crystallization trials were carried out by the hanging-drop vapour-diffusion method at 291 K using commercial screens (Index and Crystal Screens I and II) from Hampton Research. Initial drops consisted of equal volumes (1 µl) of protein solution (6.9 mg ml^{-1} in 50 m*M* Tris–HCl pH 8.0) and well solution and were equilibrated against 500 µl well solution. The initial crystallization conditions (2 *M* ammonium sulfate, 0.1 *M* bis-tris buffer pH 6.5) were optimized to obtain single crystals using 2 *M* ammonium sulfate, 2%(*v*/*v*) 2-propanol, 0.1 *M* bis-tris pH 6.5. The final drops were formed by mixing 2 µl protein solution and 2 µl well solution and produced crystals of maximum dimensions 0.7 × 0.3 × 0.3 mm (Fig. 1*a*).

However, these crystals did not yield a clean diffraction pattern (see below). An extensive screen using 36 detergents as additives was carried out around the initial crystallization conditions. The use of *n*-heptyl-β-D-thioglucoside (βHTG) resulted in a new crystal form (hexagonal prisms). Optimized crystals were obtained at 25.5 mg ml^{-1} and in drops of total volume 10 µl made up of 4 µl protein solution, 1 µl of 300 m*M* detergent solution and 5 µl well solution under the previously optimized conditions [2 *M* ammonium sulfate, 2%(*v*/*v*) 2-propanol, 0.1 *M* bis-tris pH 6.5]. Crystals reached maximum dimensions of 1.6 × 0.3 × 0.3 mm in 1–2 d (Fig. 1*b*).

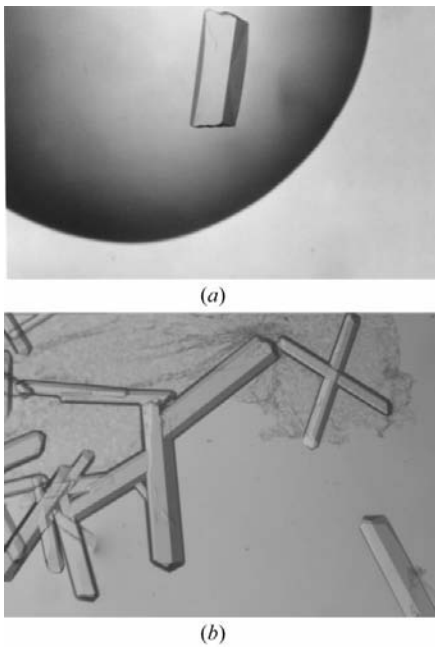

Figure 1
Effect of detergent on FPR crystals. (*a*) Orthorhombic crystals grown in 2 *M* ammonium sulfate, 0.1 *M* bis-tris buffer pH 6.5. (*b*) Trigonal crystals obtained under the same precipitant and buffer conditions but with the addition of *n*-heptyl-β-D-thioglucoside.

2.3. X-ray diffraction experiment

Preliminary diffraction data were collected on an in-house MAR Research MAR345 image-plate detector with Cu *Kα* X-rays (λ = 1.5418 Å) generated by an Enraf–Nonius rotating-anode generator equipped with a double-mirror focusing system operated at 40 kV and 90 mA. Prior to flash-cooling at 100 K, all crystals were soaked for 10 s in a cryoprotectant solution consisting of 2%(*v*/*v*) 2-propanol, 0.1 *M* bis-tris pH 6.5 and 50% saturated sodium formate solution which acts as a cryosalt (Rubinson *et al.*, 2000). The native data set from crystals obtained with detergent was subsequently collected using the synchrotron-radiation source at ESRF (Grenoble) on beamline BM16 using a MAR CCD detector, with a wavelength of 0.91835 Å. The crystal to-detector distance was set to 132.93 mm. All data were processed and scaled using the programs *MOSFLM* (Leslie, 1992) and *SCALA* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

A systematic search for crystallization conditions resulted in two crystal forms of FPR. The first crystal form diffracted to 2.1 Å resolution and belonged to the orthorhombic space group $P2_12_12$. The unit-cell parameters were *a* = 69.30, *b* = 93.58, *c* = 103.48 Å. Specific volume calculations

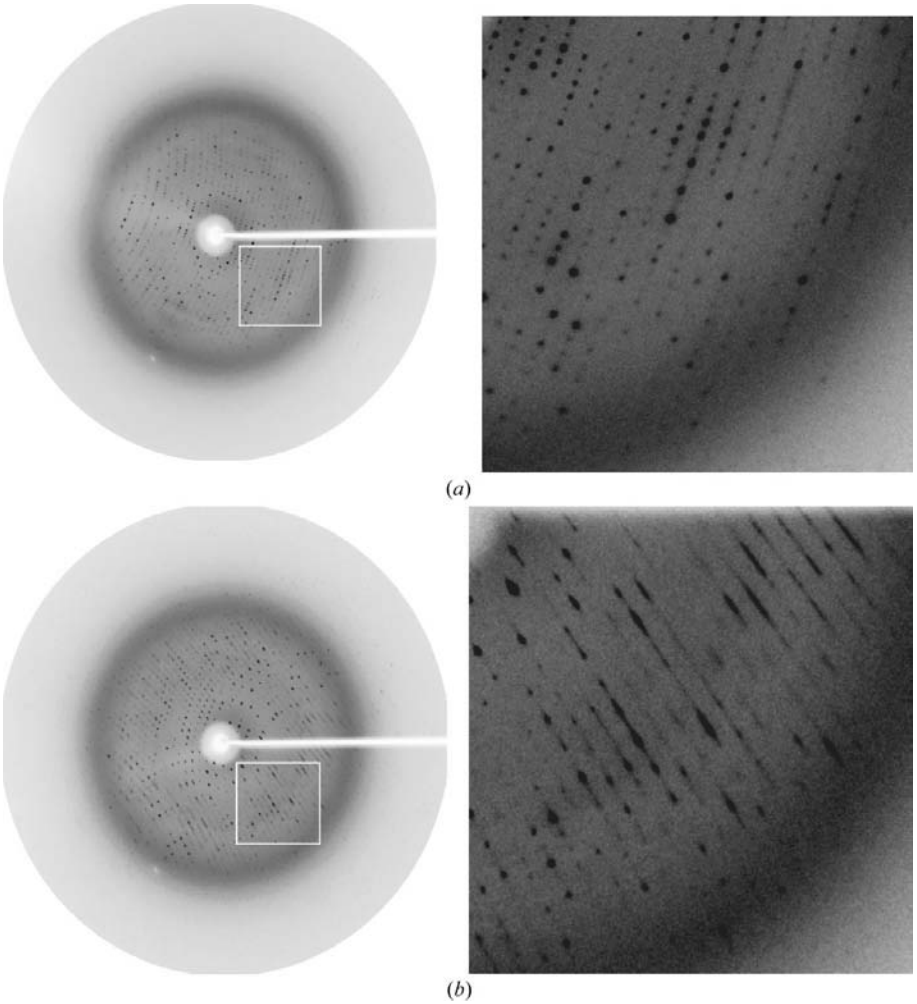

Figure 2
(*a*) X-ray diffraction pattern from orthorhombic crystals (oscillation range 1.0°). (*b*) X-ray diffraction pattern at a different rotation angle showing regions with deformed spots. Zoomed areas are highlighted.

Table 1
Data-collection statistics for FPR trigonal crystals.

Values in parentheses are for the highest resolution shell.

Crystal data	
Space group	$P3_121$
Unit-cell parameters (Å)	
a	120.5
b	120.5
c	51.1
Data collection	
Temperature (K)	100
Wavelength (Å)	0.91835
Resolution (Å)	38.9–1.8 (1.9–1.8)
Unique data	37131
Redundancy	5.9 (4.5)
Completeness (%)	86.9 (68.7)
$I/\sigma(I)$	7.1 (1.8)
R_{sym}†	0.07 (0.41)

† $R_{sym} = \sum|I - I_{av}|/\sum I$, where the summation is over symmetry-equivalent reflections.

indicated the presence of one molecule of FPR in the unit cell, with a solvent content of 56% (V_M = 2.8 Å^3 Da^{-1}). However, depending on the rotation angle the diffraction pattern showed a very substantial anisotropy (Figs. 2*a* and 2*b*). The uneven distribution of poor-quality reflections suggested that this may result from some definite disordered regions within the crystal. Processing of the X-ray data obtained from this crystal form was unsuccessful.

An intensive detergent screen was carried out on the preceding crystallization conditions to stabilize these putative disordered regions. This procedure resulted in a new crystal form that exhibited a clean and intense diffraction pattern to 1.8 Å resolution (Fig. 3). These crystals belong to the trigonal space group $P3_121$, with unit-cell parameters $a = b = 120.5$, $c = 51.1$ Å. Table 1 summarizes the statistics of X-ray data collection. The calculated Matthews coefficient (V_M = 3.56 Å^3 Da^{-1}; Matthews, 1968) estimates the presence of one protein molecule in the asymmetric unit and a solvent content of 64%.

Phasing of the diffraction data has been attempted with the molecular-replacement method using the program *AMoRe* (Navaza & Vernoslova, 1995). The full structure of FNR from *Azotobacter vinelandii* (53% sequence identity with FPR) has been used as a search model for a rotational and translational search in the 15–3.5 Å resolution range. A unique solution was obtained that after rigid-body refinement yielded a final correlation coefficient and *R* factor of 34.2% and 0.42, respectively (the next peak in the molecular replacement had a correlation coefficient of 19% and an *R* factor of 0.46). Inspection of the preliminary three-dimensional structure of FPR revealed the presence of at least three βHTG detergent molecules in the asymmetric unit. All these molecules are close to protein regions that seem to be disordered: two detergent molecules are located near a loop with a poorly defined electron-density map and a further βHTG molecule is placed near the N-terminus, for which there is no electron density for the first 14 residues. In the first case, the two βHTG molecules stabilize a completely exposed loop which protrudes into a large crystal channel (Fig. 4*a*). The third βHTG molecule, placed next to the N-terminal region, seems to increase the crystal-packing contacts with a symmetry-related molecule (Fig. 4*b*).

We can conclude here that the addition of the βHTG detergent stabilized several regions in the protein, firstly by interacting with the amino-acid chains of disordered/exposed regions and secondly by increasing the contact surface between protein mole-

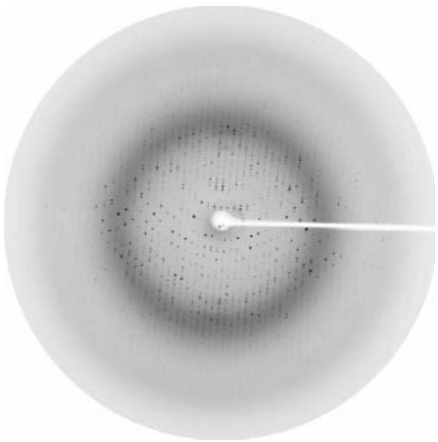

Figure 3
A typical X-ray diffraction pattern from FPR trigonal crystals grown with *n*-heptyl-β-D-thioglucoside.

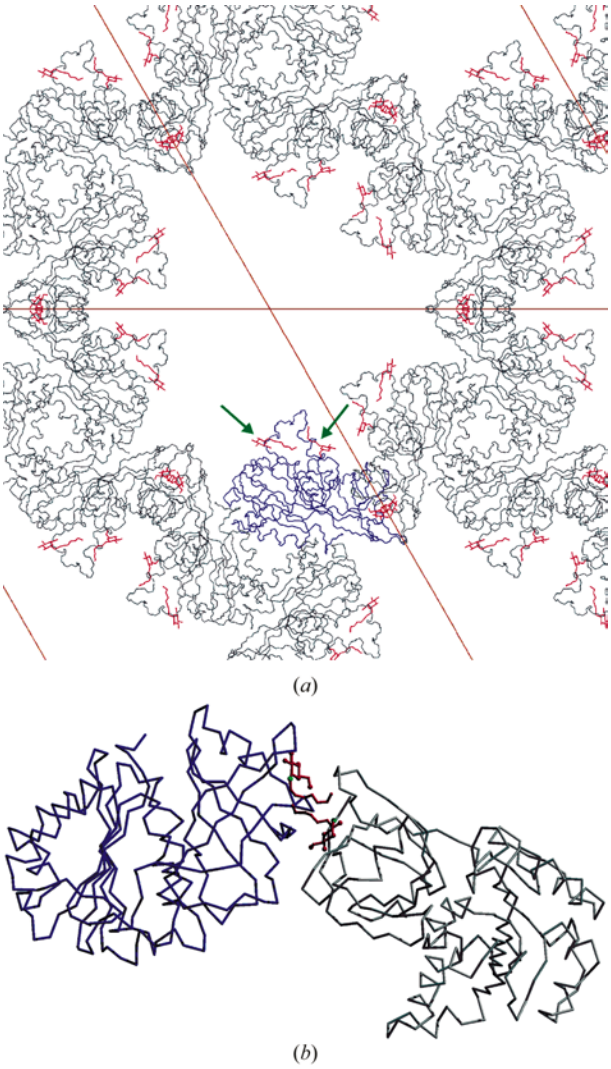

Figure 4
Crystal packing of FPR trigonal crystals. The independent FPR molecule is coloured blue and βHTG molecules are coloured red. (*a*) A general view showing the position of the detergent molecules in the crystal. The two βHTG molecules stabilizing an exposed loop (see text) are indicated by arrows. (*b*) The third detergent molecule found in the protein model stabilizes crystal contacts with a symmetry-related protein molecule.

cules. Therefore, these both effects seem to contribute cooperatively to yielding high-quality diffracting crystals. Full refinement of the three-dimensional structure of FPR is now in progress. It should provide reliable information about the electron-transfer mechanism in this enzyme and may provide insights into the physiological relevance of the disordered and exposed regions that are now stabilized by detergent molecules in the crystals.

We gratefully acknowledge the BM16 Spanish beamline at ESRF for access to synchrotron radiation and for helpful assistance during data collection. This work was supported by a grant from the Spanish Ministerio de Educación y Ciencia (BIO2002-02887) and by a contract from Bruker AXS. NC and CB acknowledge support from Consejo Nacional de Investigaciones Científicas y Técnicas (Conicet) and ANPCyT of Argentina (grant PICT-5105).

References

Arakaki, A. K., Ceccarelli, E. A. & Carrillo, N. (2000). *FASEB J.* **11**, 133–140.

Bittel, C., Tabares, L. C., Armesto, M., Carrillo, N. & Cortez, N. (2003). *FEBS Lett.* **553**, 408–412.

Carrillo, N. & Ceccarelli, A. (2003). *Eur. J. Biochem.* **270**, 1900–1915.

Ceccarelli, E. A., Arakaki, A. K., Cortez, N. & Carrillo, N. (2004). *Biochim. Biophys. Acta*, **1698**, 155–165.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Leslie, A. G. W. (1992). *Crystallographic Computing 5: From Chemistry to Biology*, edited by D. Moras, A. D. Podjarny & J. C. Thierry, pp. 39–50. Oxford University Press.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Navaza, J. & Vernoslova, E. (1995). *Acta Cryst.* A**51**, 445–449.

Rubinson, K. A., Ladner, J. E., Tordova, M. & Gilliland, G. L. (2000). *Acta Cryst.* D**56**, 996–1001.