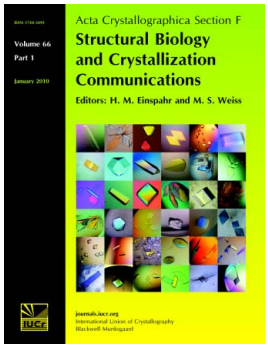
Crystallization and preliminary X-ray studies of ferredoxin-NADP + oxidoreductase encoded by Bacillus subtilis yumC

Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091
Editors: **H. M. Einspahr and M. S. Weiss**

Crystallization and preliminary X-ray studies of ferredoxin-NADP$^+$ oxidoreductase encoded by *Bacillus subtilis yumC*

Hirofumi Komori, Daisuke Seo, Takeshi Sakurai and Yoshiki Higuchi

Acta Cryst. (2010). F**66**, 301–303



Acta Crystallographica Section F: Structural Biology and Crystallization Communications is a rapid all-electronic journal, which provides a home for short communications on the crystallization and structure of biological macromolecules. It includes four categories of publication: protein structure communications; nucleic acid structure communications; structural genomics communications; and crystallization communications. Structures determined through structural genomics initiatives or from iterative studies such as those used in the pharmaceutical industry are particularly welcomed. *Section F* is essential for all those interested in structural biology including molecular biologists, biochemists, crystallization specialists, structural biologists, biophysicists, pharmacologists and other life scientists.

Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray studies of ferredoxin-NADP$^+$ oxidoreductase encoded by *Bacillus subtilis yumC*

Hirofumi Komori,[a,b] Daisuke Seo,[c] Takeshi Sakurai[c] and Yoshiki Higuchi[a,b]*

[a]Department of Life Science, Graduate School of Life Science, University of Hyogo, 3-2-1 Koto, Kamigori-cho, Ako-gun, Hyogo 678-1297, Japan, [b]RIKEN SPring-8 Center, 1-1-1 Koto, Mikazuki-cho, Sayo-gun, Hyogo 679-5248, Japan, and [c]Division of Material Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa 920-1192, Japan

Correspondence e-mail: hig@sci.u-hyogo.ac.jp

Received 11 December 2009
Accepted 4 January 2010

Ferredoxin-NADP$^+$ oxidoreductase encoded by *Bacillus subtilis yumC* has been purified and successfully crystallized in complex with NADP$^+$ in two forms. Diffraction data from crystals of these two forms were collected at resolutions of 1.8 and 1.9 Å. The former belonged to space group $P2_12_12$, with unit-cell parameters a = 63.90, b = 135.72, c = 39.19 Å, and the latter to space group $C2$, with unit-cell parameters a = 207.47, b = 64.85, c = 61.12 Å, β = 105.82°. The initial structure was determined by the molecular-replacement method using a thioredoxin reductase-like protein as a search model.

1. Introduction

Ferredoxin-NADP$^+$ oxidoreductases (FNRs) are ubiquitous flavoenzymes that contain a noncovalently bound FAD. FNR mediates the redox reaction between NADP$^+$/NADPH and ferredoxin, providing redox equivalents in cell-material synthesis (Knaff & Hirasawa, 1991; Ceccarelli *et al.*, 2004; Aliverti *et al.*, 2008). On the basis of phylogenetic and structural analyses, FNRs have been classified into two groups: plastid-type and glutathione reductase-type FNRs (Aliverti *et al.*, 2008). Both types exist as monomeric proteins composed of two nucleotide-binding domains, one for FAD binding and the other for NAD(P)H binding. Recently, it has been reported that a novel type of FNR has been obtained from several bacteria (Seo & Sakurai, 2002; Seo *et al.*, 2004; Mandai *et al.*, 2009). These FNRs occur as homodimers and share a higher level of amino-acid sequence homology with thioredoxin reductases (Waksman *et al.*, 1994) than with the two groups of FNRs mentioned above. *BLAST* searches (Altschul *et al.*, 1997) showed that a number of genes homologous to this novel FNR are present in some Gram-positive bacteria and archaea that have no gene coding for a protein with a high sequence identity to the known FNRs. This novel FNR constitutes a new group of FNRs in addition to the previously established plastid-type and glutathione reductase-type FNRs. In order to obtain the structure of this novel FNR from *Bacillus subtilis* (BsFNR), we performed its crystallization and preliminary X-ray analysis.

2. Materials and methods

2.1. Protein purification and crystallization

Expression and purification of BsFNR were carried out according to previously described methods (Seo *et al.*, 2009). Purified BsFNR was concentrated to 10 mg ml^{-1} in 50 m*M* Tris–HCl pH 8.0 buffer containing 200 m*M* NaCl. The concentration of BsFNR was determined using a molar absorption coefficient of 12 300 M^{-1} cm^{-1} at 457 nm. For crystallization of the BsFNR–NADP$^+$ complex, 2.5 m*M* NADP$^+$ was added to the BsFNR solution. Crystallization screening was carried out using Crystal Screens I and II and PEG/Ion Screen from Hampton Research and the Cryo I and II sparse-matrix crystallization screens from Emerald BioSystems with the sitting-drop vapour-diffusion method at 293 K. Protein droplets prepared by mixing 1 µl protein solution and 1 µl reservoir solution were equilibrated against 100 µl reservoir solution at 293 K.

2.2. Dynamic light scattering

Solutions of 10 mg ml^{-1} BsFNR and 2.5 m*M* NADP$^+$ were utilized for dynamic light-scattering (DLS) investigations. Debris in the protein solution was removed by centrifugation (20 000*g*, 10 min) before measurements. Automated measurements were carried out with a Zetasizer μV instrument (Malvern Instruments Ltd). A Peltier thermostatting system was used to maintain the temperature at 298 K. Size distributions were acquired at regular time intervals for BsFNR in 50 m*M* Tris–HCl buffer pH 8.0 containing 200 m*M* NaCl.

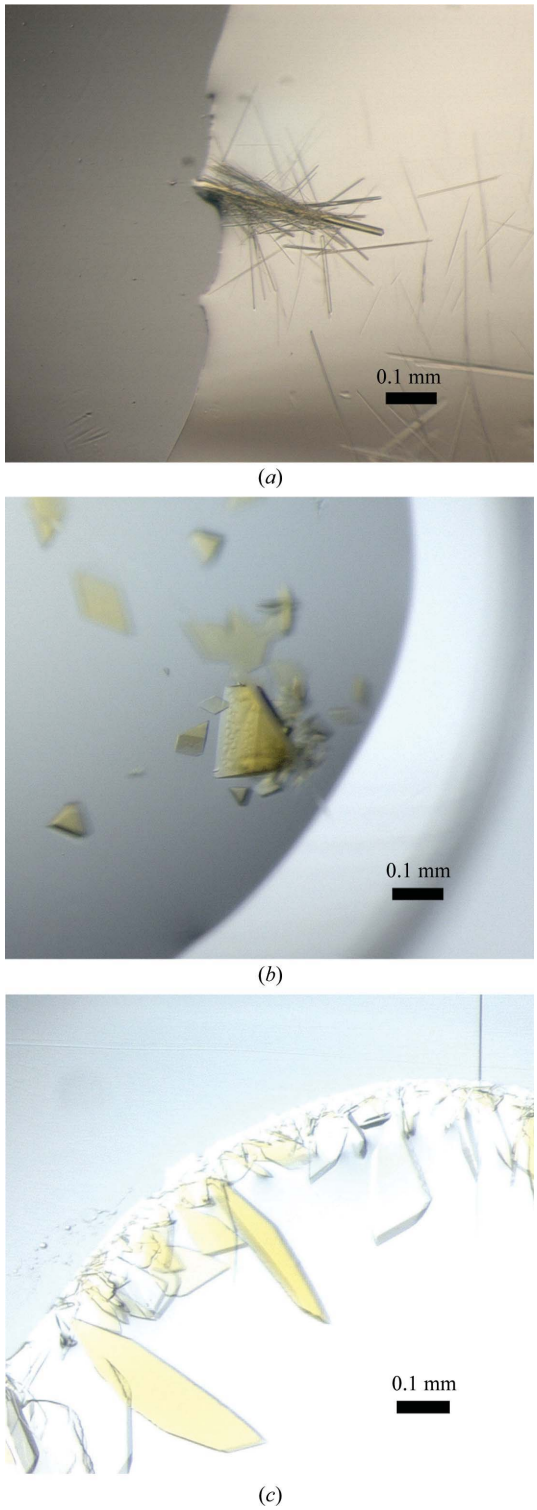


Figure 1
Crystals of *B. subtilis* FNR. (*a*) NADP$^+$-free crystals. (*b*) Form I crystals of the NADP$^+$ complex. (*c*) Form II crystals of the NADP$^+$ complex.

Table 1
Summary of crystallographic data.

	Form I	Form II
Space group	$P2_12_12$	$C2$
Unit-cell parameters (Å, °)	a = 63.90, b = 135.72, c = 39.19	a = 207.47, b = 64.85, c = 61.12, β = 105.82
Resolution (Å)	50.00–1.80 (1.86–1.80)	50.00–1.90 (1.97–1.90)
No. of unique reflections	32363	59771
Multiplicity	7.0 (6.1)	3.6 (3.0)
Completeness (%)	99.9 (99.5)	96.9 (80.4)
$\langle I/\sigma(I)\rangle$	23.0 (2.8)	22.6 (3.1)
$R_{\rm merge}$ (%)	7.1 (38.8)	5.1 (22.0)

2.3. Data collection and crystallographic analysis

X-ray diffraction data sets were collected at 100 K on beamline BL38B1 (λ = 1.0000 Å) at SPring-8 using a Rigaku Jupiter210 CCD detector. The crystal-to-detector distance was maintained at 150 mm with an oscillation range per image of 1.0°, covering a total oscillation range of 180°. Determination of the unit-cell parameters and integration of reflections were performed using the *HKL*-2000 program package (Otwinowski & Minor, 1997). Molecular replacement was carried out using the program *Phaser* (McCoy *et al.*, 2007). Further refinement and model building are in progress using the programs *REFMAC* (Murshudov *et al.*, 1997) and *Coot* (Emsley & Cowtan, 2004).

3. Results and discussion

The recombinant protein was purified to apparent homogeneity on SDS–PAGE. Its molecular mass was approximately 40 kDa on SDS–PAGE. The UV–Vis absorption spectra of the air-oxidized FNR suggested that almost all protein molecules bound a flavin. DLS was used to assess the influence of NADP$^+$ on the oligomerization of BsFNR. The deduced diameters of BsFNR without and with NADP$^+$ were 3.94 $\pm$ 0.66 and 3.90 $\pm$ 1.45 nm, respectively. The hydrodynamic radius of BsFNR was not significantly affected by the addition of NADP$^+$. The estimated molecular weight of about 80 000 indicated dimer formation. We first tried to crystallize BsFNR without NADP$^+$ by extensive screening; needle-like crystals were obtained using polyethylene glycol 3350 (PEG 3350) as precipitant (Fig. 1). However, the crystals diffracted to a resolution of approximately 10 Å and were not suitable for further crystallographic analysis. Therefore, we attempted the crystallization of BsFNR in complex with NADP$^+$ and obtained crystals with different shapes (forms I and II). The crystallization conditions of the BsFNR–NADP$^+$ complex were refined and two different crystal forms were obtained using reservoir solutions consisting of 0.1 *M* HEPES buffer pH 7.5, 30% 1,2-propanediol, 20% PEG 400 (form I) and 20% PEG 3350, 0.2 *M* sodium fluoride and 5% trehalose (form II) (Fig. 1). For data collection, a form I crystal was transferred directly from the mother liquor to a nitrogen cold stream at 100 K. A form II crystal was soaked in a cryoprotectant solution (25% PEG 3350, 0.2 *M* sodium fluoride and 15% trehalose) for a few minutes prior to freezing in the nitrogen cold stream. The diffraction of the form I and II crystals of the NADP$^+$ complex extended to 1.8 and 1.9 Å resolution, respectively. The form I crystal belonged to space group $P2_12_12$, with unit-cell parameters a = 63.90, b = 135.72, c = 39.19 Å. The form II crystal belonged to space group $C2$, with unit-cell parameters a = 207.47, b = 64.85, c = 61.12 Å,

β = 105.82°. The calculated Matthews coefficients (V_M) of 2.12 and 2.47 Å^3 Da^{-1} for forms I and II correspond to solvent contents of 42.1 and 50.3% assuming the presence of one and two protomers in the asymmetric unit, respectively, and a protein molecular weight of 40 000 (Matthews, 1968). The data-collection statistics are summarized in Table 1. Structure determination was performed by the molecular-replacement method using the structure of the thioredoxin reductase-like protein from *Thermus thermophilus* HB8 (PDB code 2zbw; A. Ebihara, M. Manzoku, Y. Fujimoto, Y. Kitamura, S. Yokoyama & S. Kuramitsu, unpublished work) as a search model (45% sequence identity). The X-ray diffraction data used to solve the BsFNR structure were collected from a form I crystal. Thioredoxin reductase consists of two globular domains (the FAD-binding and NADPH-binding domains) connected by a two-stranded β-sheet (Waksman *et al.*, 1994). Thioredoxin reductase has been proposed to undergo a conformational change on binding NADPH, leading to a rotation of the two domains by around 60° relative to each other (Lennon *et al.*, 2000). BsFNR should also consist of two domains connected by a flexible linker. In order to allow conformational flexibility of the two domains, the search model was divided into two parts (the FAD-binding and NADPH-binding domains). Molecular replacement and consequent structure refinement are now in progress.

We thank the staff at beamline BL38B1, SPring-8, Japan. This study was partly supported by the GCOE Program (YH), the Japanese Aerospace Exploration Agency Project (YH), the Sumitomo Foundation (HK), the Hyogo Science and Technology Association (HK) and Grants-in-Aid for Scientific Research (18GS0207; YH) and for Young Scientists B (HK) from the Japan Society for the Promotion of Science.

References

Aliverti, A., Pandini, V., Pennati, A., de Rosa, M. & Zanetti, G. (2008). *Arch. Biochem. Biophys.* **474**, 283–291.
Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Miller, W. & Lipman, D. J. (1997). *Nucleic Acids Res.* **25**, 3389–3402.
Ceccarelli, E. A., Arakai, A. K., Cortez, N. & Carrill, N. (2004). *Biochim. Biophys. Acta*, **1698**, 155–165.
Emsley, P. & Cowtan, K. (2004). *Acta Cryst.* D**60**, 2126–2132.
Knaff, D. B. & Hirasawa, M. (1991). *Biochim. Biophys. Acta*, **1056**, 93–125.
Lennon, B. W., Williams, C. H. Jr & Ludwig, M. L. (2000). *Science*, **289**, 1190–1194.
Mandai, T., Fujiwara, S. & Inaoka, S. (2009). *FEBS J.* **276**, 2416–2429.
Matthews, B. W. (1968). *J. Biol. Chem.* **33**, 491–497.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
Murshudov, G. N., Vagin, A. A. & Dodson, E. J. (1997). *Acta Cryst.* D**53**, 240–255.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Seo, D., Kamino, K., Inoue, K. & Sakurai, H. (2004). *Arch. Microbiol.* **182**, 80–89.
Seo, D., Okabe, S., Yanase, M., Kataoka, K. & Sakurai, T. (2009). *Biochim. Biophys. Acta*, **1794**, 594–601.
Seo, D. & Sakurai, H. (2002). *Biochim. Biophys. Acta*, **1597**, 123–132.
Waksman, G., Krishna, T. S., Williams, C. H. Jr & Kuriyan, J. (1994). *J. Mol. Biol.* **236**, 800–816.