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Purification, crystallization and preliminary X-ray diffraction of fluorescence recovery protein from *Synechocystis* PCC 6803

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Fluorescence recovery protein (FRP), which is encoded by the *slr1964* gene in *Synechocystis* PCC 6803, plays a key role in the orange carotenoid protein-related photoprotective mechanism in cyanobacteria. As the crystal structure of FRP may provide information about the biological functions and mechanism of action of the protein, recombinant full-length FRP and a truncated form were overexpressed, purified and crystallized at 291 K using ethylene imine polymer as the precipitant. An FRP data set was collected to a resolution of 2.75 Å at low temperature (100 K). The crystal belonged to space group $P4_12_12$, with unit-cell parameters $a = b = 61.9$, $c = 160.7$ Å, $\alpha = \beta = \gamma = 90^\circ$. Assuming that the asymmetric unit contains three molecules, the Matthews coefficient was calculated to be 2.1 Å^3 Da^{-1}.

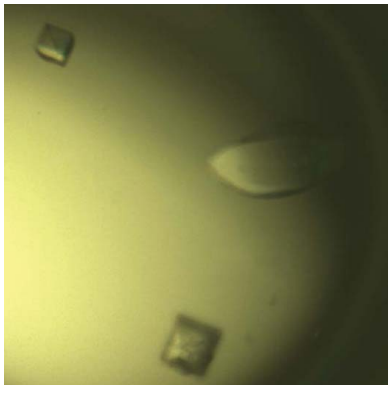
1. Introduction

Although light is essential for photosynthetic organisms, too much light can be harmful. Photoprotective mechanisms have evolved to protect organisms from too much light. One such mechanism involves fluorescence recovery protein (FRP), which is encoded by the *slr1964* gene in *Synechocystis* PCC 6803. FRP plays a key role in the orange carotenoid protein-related photoprotective mechanism in cyanobacteria (Boulay, Wilson *et al.*, 2008).

Orange carotenoid protein (OCP) is a photoactive protein that is involved in a photoprotective mechanism associated with the phycobilisome (PB). The PB is a large extrinsic membrane complex that includes many types of chromophorylated phycobiliproteins and linker peptides that allow the absorption and unidirectional transfer of light energy to chlorophyll *a* of photosystem II in the dark (Wilson *et al.*, 2006; Adir, 2005). This photoprotective mechanism has been reported to involve an OCP-triggered reduction in energy transfer between the photosynthetic antenna and the reaction centres (El Bissati *et al.*, 2000). When OCP is activated by blue-green light it is converted to an unstable red form (OCP^r) which binds strongly to the PB. In the dark, OCP stays in a stable form (OCP^o) which binds to the PB only weakly. When OCP^o absorbs light it is transformed into the OCP^r form, which subsequently acts as a fluorescence quencher after its interaction with the PB. Boulay and coworkers, who were the first to identify and overexpress FRP, showed that it mediates the recovery of full antenna capacity when light decreases (Boulay, Abasova *et al.*, 2008). FRP is believed to bind to the N-terminus of OCP^r and to help to separate OCP^r from the PB. This binding interaction promotes the conversion of OCP^r to OCP^o and assists in inducing the recovery of fluorescence (Boulay, Abasova *et al.*, 2008).

The Slr1964-like protein FRP contains 106–111 amino-acid residues (14 kDa). Its structure has been predicted to consist of one long helix and three short α-helices (Sato *et al.*, 2007). Size-exclusion chromatography showed that FRP exists as a stable trimeric complex and FRP appears to be a membrane protein or to be persistently bound to the membrane as it is only detected in the membrane fraction (Boulay, Abasova *et al.*, 2008).

Here, we describe the purification and crystallization of FRP. To our knowledge, this is the first reported crystallization of an FRP from a cyanobacterium.



2. Protein expression and purification

The full-length *slr1964* gene from *Synechocystis* PCC 6803 encoding FRP was synthesized and cloned as a *Bam*HI–*Xho*I fragment into pET-28a vector (Novagen) with an N-terminal 6×His tag and the protein was overexpressed in *Escherichia coli* strain BL21 (DE3) (Novagen). A truncated form of FRP (Met26–Leu134) was amplified and inserted into the same vector with *Bam*HI and *Xho*I sites. The forward and reverse PCR primers used for amplification were 5′-CCGGGATCCATGTTACAAACCGCCGAAGC-3′ and 5′-GC-CTCGGTTATCACAGCCGGGCCAGGG-3′, respectively. The accuracy of the inserts was verified by sequencing. The recombinant bacteria were grown in LB medium at 310 K. The constructs were expressed and purified as described below for full-length FRP.

When the optical density of the culture at 600 nm reached 0.4–0.6, the culture was cooled to 289 K and supplemented with isopropyl β-D-1-thiogalactopyranoside to a final concentration of 0.1 m*M*. After an overnight induction period, the cells were harvested by centrifugation at 4500*g* for 20 min at 277 K. The cell pellets were resuspended in lysis buffer (20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, and 10 m*M* imidazole) and disrupted by sonication. The cell debris was removed by centrifugation at 14 400*g* for 30 min at 277 K.

The supernatant was collected and loaded onto Ni^{2+}–NTA affinity resin (Qiagen) and the column was washed with wash buffer (20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 20 m*M* imidazole). The fusion protein was eluted with lysis buffer containing 100 m*M* imidazole and the eluate was dialyzed against 20 m*M* Tris–HCl pH 8.0, 50 m*M* NaCl and 20 U thrombin overnight at 277 K. The resulting mixture was reloaded onto the Ni^{2+}–NTA column for removal of the cleaved N-terminal His tag.

The protein was further purified by anion-exchange chromatography on a Resource Q column (GE Healthcare) equilibrated in 20 m*M* Tris–HCl pH 8.0 and eluted with a 0–1000 m*M* NaCl gradient. The peak fraction was concentrated to about 10 mg ml^{-1} and applied onto Superdex 200 10/300 GL (GE Healthcare) in a buffer consisting of 20 m*M* Tris–HCl pH 8.0 and 150 m*M* NaCl. The peak fraction that contained protein at >95% purity (as indicated by SDS–PAGE) was collected and concentrated to 20 mg ml^{-1} for crystallization.

Simultaneously, we tried another expression method. The truncated FRP (Met26–Leu134) was also subcloned into pGEX-6P-1 vector with an N-terminal glutathione *S*-transferase (GST) using the *Bam*HI and *Xho*I restriction sites. The transformed *E. coli* BL21 (DE3) (Novagen) cells were induced with 0.1 m*M* isopropyl β-D-1-thiogalactopyranoside to an optical density (600 nm) of 0.6. After growth for 8 h at 289 K, the cells were harvested, resuspended in 1× phosphate-buffered saline (140 m*M* NaCl, 2.7 m*M* KCl, 10 m*M* Na_2HPO_4, 1.8 m*M* KH_2PO_4 pH 7.3) and homogenized with a French press (JNBIO, Guangzhou, People's Republic of China; two passes at 103–138 MPa). Cell debris was removed by high-speed centrifugation for 10 min at 18 000*g*.

The supernatant was collected and the fusion protein was purified by Glutathione Sepharose 4B affinity chromatography (GE Healthcare). The GST tag of the fusion protein was cleaved with PreScission Protease (GE Healthcare) overnight at 277 K. The following day, the target protein was eluted with 1× phosphate-buffered saline and further purified as described previously for the His-tagged protein.

Although the purified His- and GST-tagged proteins were both pure and stable for crystallization, the His-tag fusion system afforded a better yield. More than 5 mg of the His-tagged target protein was obtained from each litre of culture, whereas only 2–3 mg of the GST-tagged protein was obtained. Therefore, we used the FRP proteins from the His-tag expression vector for our crystallographic studies.

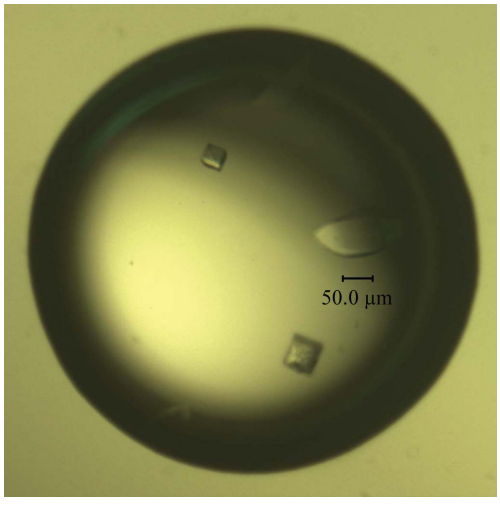


Figure 1
Optimized crystals of truncated FRP.

Table 1
Data-collection and processing statistics for truncated FRP.

Values in parentheses are for the highest resolution shell.

Space group	$P4_12_12$
Unit-cell parameters (Å, °)	$a = b = 61.9$, $c = 160.7$, $\alpha = \beta = \gamma = 90$
Wavelength (Å)	1.0000
Resolution range (Å)	50.0–2.75 (2.90–2.75)
Total reflections	306133 (22113)
Unique reflections	32463 (3152)
Average $I/\sigma(I)$	15.6 (9.0)
R_{merge}† (%)	6.2 (33.7)
Data completeness (%)	98.7 (98.2)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the mean intensity of the observations $I_i(hkl)$ of reflection *hkl*.

3. Crystallization

We determined initial crystallization conditions for the truncated forms of FRP using commercially available crystal screening kits (Index and Crystal Screen 2 from Hampton Research) with the hanging-drop vapour-diffusion method. Droplets consisting of 1 µl protein solution (20 mg ml^{-1}) and 1 µl mother liquor were equilibrated against 200 µl reservoir solution at 289 K.

After 3 d, needle-shaped and rectangular crystals of truncated FRP of various sizes were obtained using several conditions (Index condition No. 9 and Crystal Screen 2 condition Nos. 16 and 37). All tested crystals belonged to the same tetragonal space group ($P4_12_12$). The initial small crystals showed diffraction to ∼3.5 Å resolution but with significant twinning. After extensive optimization, a good single crystal with dimensions of 200 × 100 × 50 µm (Fig. 1) was obtained from 400 m*M* NaCl, 150 m*M* sodium citrate tribasic dihydrate pH 5.5 and 5% ethylene imine polymer. This crystal diffracted to at least 2.75 Å resolution. Data-collection and processing statistics are given in Table 1. Only a few small crystals of the full-length protein were obtained, but none of the crystals showed diffraction beyond 5 Å resolution.

4. X-ray diffraction analysis

The rectangular truncated FRP crystals were transferred to a solution consisting of mother liquor supplemented with 5–10%(*v*/*v*) glycerol,

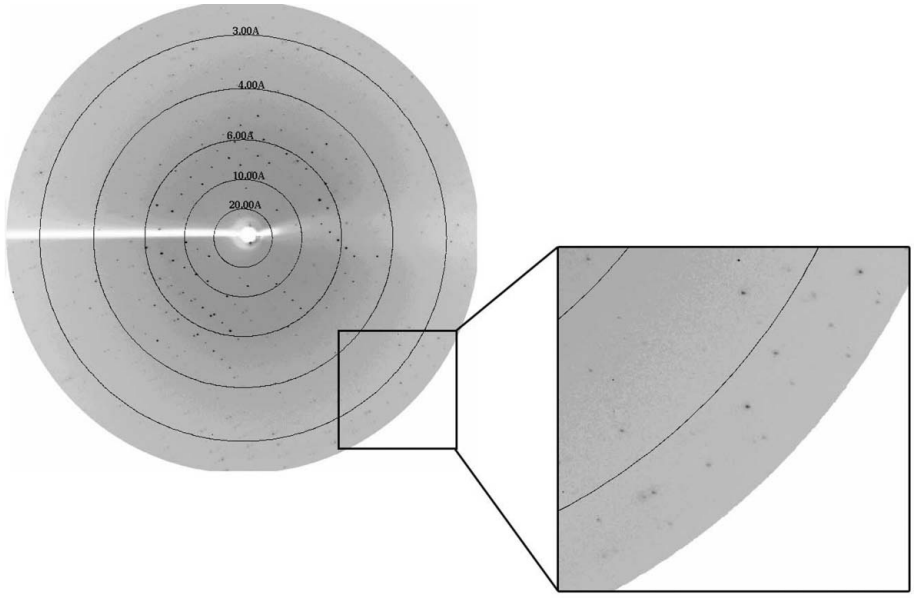


Figure 2
Diffraction pattern of truncated FRP. The exposure time was 30 s, the crystal-to-detector distance was 224 mm and the oscillation range per frame was 0.5°. The diffraction image was collected on a MAR165 CCD detector. An enlarged image is shown on the right.

harvested with a nylon loop and cooled directly in a nitrogen stream at 100 K. X-ray diffraction data were collected using a MAR165 CCD detector on beamline 3W1A at the Beijing Synchrotron Radiation Facility at a wavelength of 1.0000 Å. The crystal belonged to space group $P4_12_12$, with unit-cell parameters $a = b = 61.9$, $c = 160.7$ Å, $\alpha = \beta = \gamma = 90^\circ$ (Fig. 2). Assuming that the asymmetric unit consists of three molecules, the Matthews coefficient was calculated to be 2.1 Å^3 Da^{-1} with a solvent content of 40.6% (Matthews, 1968). The raw data were processed with the *HKL*-2000 software (Otwinowski & Minor, 1997). Because no structural homologues of FRP have been reported, we initially tried to solve the FRP crystal structure by molecular replacement using *Phaser* (McCoy *et al.*, 2007) with a model (PDB entry 3bn0; Wallgren *et al.*, 2008) obtained using *BLAST* with the FRP sequence as the search molecule against the Protein Data Bank (Lu *et al.*, 2009). Unfortunately, no solutions were found. We are currently producing heavy-atom derivatives and selenomethionine-substituted protein in order to determine the phases of the diffraction data.

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