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Crystallization and preliminary X-ray analysis of plastocyanin from cyanobacterium *Synechococcus* sp. PCC 7942

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A plastocyanin from the cyanobacterium *Synechococcus* sp. PCC7942 has been crystallized in two different forms by hanging-drop vapour diffusion with ammonium sulfate as precipitant. Form I is hexagonal, space group $P6_1$ or $P6_5$, with unit-cell dimensions $a = b = 34.62$ and $c = 107.22$ Å. Form II is tetragonal, space group $P4_1$ or $P4_3$, with unit-cell dimensions $a = b = 43.05$ and $c = 56.94$ Å. Form I crystals diffract to 2.5 Å using graphite-monochromated Cu $K\alpha$ radiation from a Rigaku RU-300 rotating-anode generator operated at 40 kV and 100 mA. Form II crystals diffract to 1.9 Å using synchrotron radiation at beamline BL6A of the Photon Factory (KEK). Molecular-replacement calculations using the structure of plastocyanin from *Ulva pertusa* have been performed.

1. Introduction

Plastocyanin is a blue copper protein which functions as an electron carrier between cytochrome b_6f complex and $P700^+$ of photosystem I. Plastocyanin is the sole component in the photosynthetic electron-transfer system in higher plants; however, cytochrome c_6 is known to be an alternative electron carrier in cyanobacteria and some green algae (Aitken, 1976; Sandmann *et al.*, 1983; Sandmann, 1986).

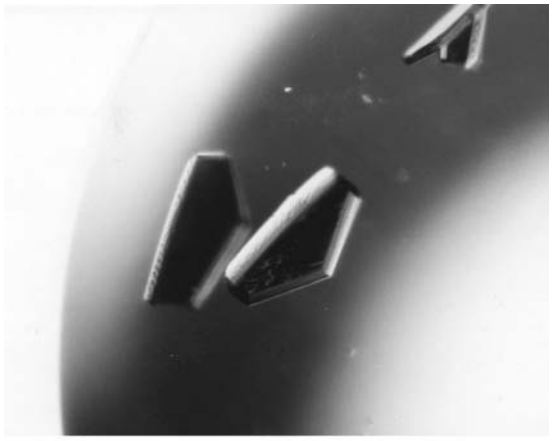
The cyanobacterium *Synechococcus* sp. PCC7942 was thought to be plastocyanin deficient, owing to the failure to detect plastocyanin in immunological studies (Aitken, 1976; Van der Plas *et al.*, 1989; Geerts *et al.*, 1994). Recently, however, the *petE* gene in *Synechococcus* encoding plastocyanin was cloned (Clarke & Campbell, 1996). The plastocyanin gene of *Synechococcus* sp. PCC7942 has been expressed in *E. coli*, and the protein is correctly processed as a mature protein molecule (Suzuki *et al.*, 1999).

Binding sites and electron-transfer kinetics have been investigated, and two distinct surface patches for exchange of electrons with the physiological redox partners are proposed from using small inorganic complexes or the electron-transfer proteins cytochrome *f* and $P700^+$. One is the hydrophobic patch around the solvent-exposed His87 and the other is the negatively charged acidic patch around Tyr83. The latter consists of two acidic clusters at positions 42–45 and 59–61. In the algal plastocyanins, the residues at positions 58 and 59 are deleted relative to the plant protein. Instead, the algal plastocyanins have two negatively charged residues, Asp53 and either Asp85 or Glu85 (Gross, 1996; Navarro *et al.*, 1997). The primary structure of *Synechococcus* plastocyanin indicates a total of 91 amino-acid residues with deletions at positions 43–51 relative to the plant protein and the acidic residues around Tyr83 replaced by neutral or positive amino-acid residues. Such replacement of amino-acid residues is general for other cyanobacterial plastocyanins (Redinbo *et al.*, 1994; Navarro *et al.*, 1997).

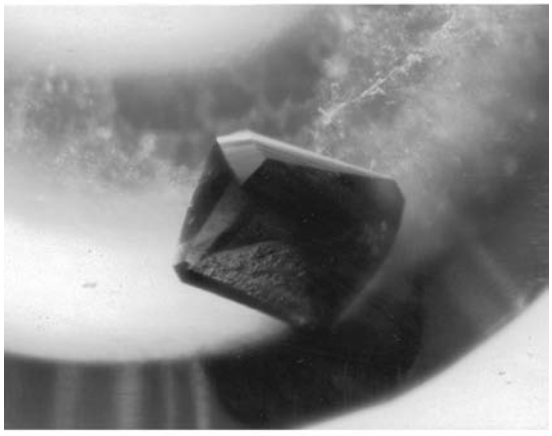
Here, we report the crystallization and preliminary X-ray diffraction studies of *Synechococcus* sp. PCC 7942 plastocyanin in the oxidized form.

2. Crystallization

Recombinant sPC was expressed in *Escherichia coli* and purified as described (Suzuki *et al.*, 1999). Crystallization experiments were performed at 293 K using the hanging-drop vapour-diffusion method (McPherson, 1982). The drops consisted of 2 µl protein solution in oxidized form and 2 µl precipitating solution suspended over a 0.5 ml reservoir containing the same precipitating solution. Screening for crystallization conditions was carried out using ammonium sulfate as precipitant, as reported previously for crystallization of plastocyanins from other species (Li *et al.*, 1997; Yoshizaki *et al.*, 1981). Two different crystals grew under similar conditions at pH 5.3 (forms I and II). Thin plate-like crystals ($0.2 \times 0.1 \times 0.05$ mm) were obtained at room temperature after 1–2 d in 1.6–1.8 *M* ammonium sulfate when the protein was concentrated to 20–40 mg ml^{-1} in



(*a*)



(*b*)

Figure 1
Crystals of plasytocyanin from *Synechococcus* sp. PCC7942. Two lattice types have been observed: (*a*) form I, hexagonal; (*b*) form II, tetragonal.

Milli-Q water. Macroseeding (Stura & Wilson, 1991) was employed by transferring thin crystals to crystal-free drops. Thus, macrocrystals (0.4 × 0.3 × 0.1 mm) could be grown in a day (form I; Fig. 1*a*). Alternatively, when the protein was concentrated to 40–120 mg ml^{-1}, large crystals (0.5 × 0.5 × 0.3 mm) were obtained after two weeks in 1.8–2.0 *M* ammonium sulfate (form II; Fig. 1*b*).

3. X-ray diffraction study

Crystals of sPC were mounted in quartz capillaries and subjected to X-ray diffraction. X-ray diffraction experiments on form I crystals were carried out using an R-AXIS IIc imaging-plate area detector mounted on a Rigaku RU-300 rotating-anode source operating at 40 kV, 100 mA with Cu $K\alpha$ radiation. The data sets were indexed and integrated using *DENZO* (Otwinowski, 1993). The space group of the form I sPC crystal was determined to be hexagonal $P6_1$ or $P6_5$ with unit-cell parameters $a = b = 34.6$ and $c = 107.2$ Å. The integrated intensities were scaled and reduced using *SCALEPACK* (Otwinowski, 1993). The data from form I crystals are 99.0% complete in the range 15–2.5 Å, with an overall $R_{\rm merge}$ of 16.8%.

Data collection for form II crystals was performed on beamline BL6A of the Photon Factory (KEK) using the screenless Weissenberg camera (Sakabe, 1991). The space group was determined to be tetragonal $P4_1$ or $P4_3$ with unit-cell parameters $a = b = 43.1$ and $c = 56.9$ Å. Assuming one molecule of sPC in the asymmetric unit, the value of Matthews constant V_m (Matthews, 1968) is 2.70 Å^3 Da^{-1}, corresponding to a solvent content of 54% which is a normal value for protein crystals. The final data from form II crystals yielded 8314 independent reflections and was 99.1% complete from 39058 total reflections in the range 30–1.9 Å, with an overall $R_{\rm merge}$ of 7.1%. The highest resolution shell (1.97–1.90 Å) was 97.3% complete and had an $R_{\rm merge}$ of 12.3%.

Preliminary molecular-replacement calculations on form II crystal data were performed with *X-PLOR* (Brünger, 1990). The search model was the plastocyanin structure from *Ulva pertusa* (PDB code 1IUZ; Shibata *et al.*, 1999). Assuming that the space group was $P4_1$, a clear peak was found with a Tf value of 0.387 and an *R* factor of 46.6% (15–4 Å) after translation-function calculations. The *R* factor was reduced to 41.9% (8–3 Å) using rigid-body refinement. Inspection of the crystal packing using *O* (Jones *et al.*, 1991) is currently in progress.

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