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Crystallization and preliminary crystallographic studies on the extrinsic 23 kDa protein in the oxygen-evolving complex of photosystem II

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The extrinsic 23 kDa protein in the oxygen-evolving complex (OEC23 or PsbP) of photosystem II (PS II) helps to retain calcium and chloride ions, which are essential cofactors for the photosynthetic evolution of oxygen. OEC23 is found in the PS II complex from higher plants and green algae, but not in that from cyanobacteria or other ancestral photosynthetic organisms. In order to elucidate the three-dimensional structure and its molecular evolution, OEC23 was crystallized by the hanging-drop vapour-diffusion technique with PEG 4000 as a precipitant. Diffraction data to a resolution of 2.0 Å were collected from a native crystal belonging to space group $P2_12_12$, with unit-cell parameters a = 91.36, b = 74.15, c = 52.16 Å.

1. Introduction

The photosynthetic oxygen-evolving complex (OEC) in photosystem II (PS II) participates in the use of strong oxidants produced under light to oxidize water, forming molecular oxygen as a byproduct. This process is catalyzed by an Mn_4 cluster surrounded by at least three extrinsic subunits bound to the lumenal surface of the PS II complex (for a review, see Hankamer *et al.*, 1997). In oxygenic photosynthetic organisms of all types, OEC33 (named after its apparent molecular mass) encoded by the *psb*O gene stabilizes the Mn_4 cluster. Higher plants and green algae have two other extrinsic proteins: OEC23 (PsbP) and OEC17 (PsbQ) (for a review, see Seidler, 1996). These two polypeptides help to retain Ca^{2+} and Cl^-, which are essential cofactors in the water-splitting reaction (Ghanotakis *et al.*, 1984; Miyao & Murata, 1984). On the other hand, cyanobacteria and red algae contain cyt c_{550} (PsbU) and 12 kDa protein (PsbV) instead of OEC23 and OEC17 (Shen *et al.*, 1992). Functionally, cyt c_{550} and 12 kDa protein resemble OEC23 and OEC17, but they differ from OEC23 and OEC17 with regard to both their primary sequences and their binding to the PS II complex (Shen & Inoue, 1993; Enami *et al.*, 2000 and references cited therein).

Recently, the crystal structures of PS II complexes from thermophilic cyanobacteria have been reported at resolutions of 3.8 Å (Zouni *et al.*, 2001) and 3.7 Å (Kamiya & Shen, 2003). The crystal structure of cyt c_{550} has also been reported (Frazao *et al.*, 2001; Sawaya *et al.*, 2001). However, there is still limited information available regarding the structure of the PS II complex from higher plants and especially of the oxygen-evolving complex.

We report here the first crystallization of OEC23. Diffraction data were collected to a resolution of 2.0 Å from a native crystal belonging to space group $P2_12_12$, with unit-cell parameters a = 91.36, b = 74.15, c = 52.16 Å.

2. Materials and methods

2.1. Expression and purification

Recombinant OEC23s (from spinach, cucumber and tobacco) were expressed in *Escherichia coli* and purified as described previously (Ifuku & Sato, 2001, 2002). Purified protein was desalted through a PD-10 column (Amersham Biosciences) that had been pre-equilibrated with sterile distilled water. Protein solution was concentrated using a Centricon-10 (Millipore). The protein was concentrated to 10–20 mg ml^{-1}, frozen in liquid nitrogen and stored at 193 K.

2.2. Crystallization

All crystallization trials were performed using the hanging-drop vapour-diffusion method at 277, 293 and 298 K. The initial experiment to determine the crystallization conditions was performed with Crystal Screen and Crystal Screen II (Hampton Research) by mixing 2 µl of protein solution (10–20 mg ml^{-1} protein) with 2 µl of various reservoir solutions and equilibrating the drops over 1 ml of their respective reservoirs. The first screening showed that PEG 4000 and 6000 were effective precipitants for the crystallization of OEC23. The conditions were further optimized by varying the concentration of proteins, additives and the buffer systems, including the concentration and pH.

2.3. Data collection and processing

The crystals were flash-frozen in liquid nitrogen with a cryoprotectant solution

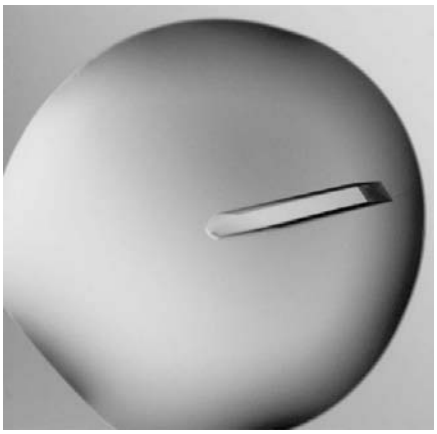

Figure 1
A typical crystal of OEC23 from *Nicotiana tabacum*. The dimensions of the crystal are approximately 0.4 × 0.8 × 0.2 mm.

containing 50 m*M* MES pH 6.5, 0.1 *M* lithium sulfate, 35%(*w*/*v*) PEG 4000, 5%(*w*/*v*) glucose and 5%(*w*/*v*) sucrose. X-ray diffraction data were collected from the OEC23 crystals at 100 K using an R-AXIS IV image-plate area detector and Cu $K\alpha$ radiation from a Rigaku FR-D rotating-anode generator operating at 50 kV and 60 mA. Oscillation images were processed and scaled using *CrystalClear* software (Rigaku/MSC; Pflugrath, 1999).

3. Results and discussion

Although the OEC23s of several plant species were tested, only tobacco OEC23 (2AF) formed a three-dimensional crystal. SDS–PAGE of the dissolved crystal showed that it contained both mature protein and partially degraded protein (data not shown). The partial degradation of OEC23 has been reported previously: nine amino-terminal residues of mature OEC23 are easily degraded by protease and are dispensable for its function (Miyao *et al.*, 1988). Since heterogeneous degradation of the protein may increase the mosaicity of the crystal, the DNA sequence encoding the nine amino-terminal residues of tobacco OEC23 was deleted from the gene (GenBank accession No. X62427). A truncated OEC23 was expressed in *E. coli* and crystallized under the same conditions as the full-length protein.

Table 1
Summary of crystal parameters and data-collection statistics.

Values in parentheses are for the highest resolution shell (2.07–2.0 Å).

Resolution (Å)	2.0
Space group	$P2_12_12$
Unit-cell parameters	
a (Å)	90.36
b (Å)	74.15
c (Å)	52.16
$R_{\rm merge}$ (%)	3.9 (8.5)
$I/\sigma(I)$	15.8 (8.2)
No. of observed reflections	86516
No. of unique reflections	24255
Completeness (%)	99.3 (99.3)

Optimization of crystallization conditions revealed that addition of 0.1 *M* lithium sulfate or 0.1 *M* ammonium sulfate improved the quality of the crystals. The tobacco OEC23 crystal used for X-ray data collection (Fig. 1) was formed under the following conditions. Protein solution (10 mg ml^{-1} OEC23) was mixed with reservoir solution [50 m*M* MES pH 6.5, 0.1 *M* lithium sulfate and 28%(*w*/*v*) PEG 4000] in a 1:1 ratio and the resulting 4 µl drops were equilibrated at 298 K for two weeks.

The 2.0 Å data set had an $R_{\rm merge}$ = 3.9% and a completeness of 99.3%. The unit cell was orthorhombic, space group $P2_12_12$, with unit-cell parameters a = 91.36, b = 74.15, c = 52.16 Å. Detailed crystal parameters and data-collection statistics are shown in Table 1. Assuming that two OEC23 molecules are present in the asymmetric unit, the specific volume $V_{\rm M}$ was 2.26 Å^3 Da^{-1}, corresponding to a solvent content of 45%.

Successful crystallization and initial data collection are the first steps in determining the three-dimensional structure of OEC23. The structure of OEC23 should provide a deeper insight into not only the molecular evolution of this protein, but also the unknown functions of the many OEC23-related proteins that have recently been found in the thylakoid lumen of higher plants (Peltier *et al.*, 2002). We have already identified a promising heavy-atom derivative to address the phase problem. The structure will be solved by multi-wavelength anomalous dispersion (MAD) experiments using synchrotron X-ray radiation at SPring-8 (Hyogo, Japan).

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