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Fitting of a two-domain model for PsbO into a 3D map of the PSII-LHCII supercomplex obtained by electron cryo-microscopy

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Introduction

Photosystem II (PSII) is a multi-subunit transmembrane protein complex (Hankamer et al., 1997) that utilises the energy of visible light to break the O-H bond of water and produce molecular oxygen. The redox active cofactors of PSII are bound to the reaction centre proteins D1 and D2, arranged in such a way that electron flow occurs across the membrane, with water oxidation occurring on the luminal/periplasmic surface. The catalytic site for water oxidation consists of a (Mn)₄ cluster, ligated to a tyrosine on the D1 protein, named Y_Z (Debus, 1992). This inorganic group is stabilized by an extrinsic 33 kDa protein (PsbO) encoded by the *psbO* gene. In higher plants, the PsbO is joined by at least two other extrinsic proteins, having apparent molecular weights of 23 kDa (PsbP) and 17 kDa (PsbQ), which all together form the Oxygen Evolving Complex (OEC) (Barber et al., 1997). Little is known about the structure of these higher plant/algal OEC proteins (Seidler, 1996), or about their specific location and arrangement in relation to the intrinsic subunits of PSII. The secondary structure of PsbO has been probed by Fourier Transform Infra Red (FTIR) spectroscopy indicating that it is predominantly of β -structure (He, 1991), a conclusion reinforced by circular dichroism (Xu et al., 1994). Structural predictions also suggest that it is a β -protein consisting of 9 or more β -strands with a high proportion of loops and turns (Bricker and Frankel, 1998). Studies of PSII using electron microscopy (EM) and single particle analyses have provided 3D structural information for the OEC proteins of spinach (Nield et al., 2000a), *Chlamydomonas reinhardtii* and *Synechococcus elongatus* (Nield et al., 2000b). In the former, the 3D structure was obtained using vitrified samples at 24 Å resolution, and in all cases the OEC proteins were observed as electron densities extending from the membrane surface on the luminal side.

The spinach PSII supercomplex 3D map has been refined to 17Å and here we focus on the extrinsic regions located on the luminal side of PSII and assignment of the PsbO protein in particular. We have carried out a series of EM-based structural studies on biochemically-washed spinach PSII-LHCII supercomplex preparations, coupling these with computational protein comparative analyses and structural predictions, allowing for a remote 3D model for PsbO to be constructed. This information was combined with the various EM density maps obtained to indicate the specific location and stoichiometry of the PsbO extrinsic protein within the spinach OEC complex.

Materials and methods

A number of spinach PSII-LHCII supercomplex preparations were isolated by sucrose density gradient centrifugation of solubilised PSII-enriched membranes (BBYs) (Nield et al., 2000a). The PSII-enriched membranes were previously either left untreated or washed with 1 M NaCl, to remove the PsbP / PsbQ extrinsic polypeptides, or with 1 M Tris, to remove all extrinsic polypeptides (Miyao and Murata, 1984). For the first part of our structural studies, electron cryo-microscopy was performed on an untreated supercomplex preparation using a Philips CM200 FEG electron microscope (EM) at liquid nitrogen temperature. The Imagic-V software environment was used to image process a ~15,700 single particle dataset, built from all possible single particles observed in 108 micrographs. This dataset was then corrected for the contrast transfer function (CTF) and processed by reference free alignment and multivariate statistical analysis (see Nield et al., 2000a). Computer purification techniques identified a number of particle sub-populations differing in size and shape. These were then processed independently. The two main populations, after processing, allowed for the calculation of two independent 3D maps by angular reconstitution (Schatz et al., 1995), one map displaying the most extrinsic mass observed, the other with hardly any extrinsic mass. For the second part of the studies, all three supercomplex preparations (untreated/NaCl-washed/Tris-washed) were negatively stained and imaged at room temperature on a Philips CM100 EM. 5 micrographs of each preparation were used to build three non-CTF corrected single particle datasets, where the first minima of their CTF was ~20 Å. Independently, each of these ~1,000 particle datasets was subjected to reference-free alignment and classification procedures as above, identifying mainly top and side view class averages of the PSII-LHCII supercomplex. In particular, after iterative refinement, improved side view class averages for each preparation allowed for the size and distribution of the extrinsic luminal mass to be compared.

Results

SDS-PAGE and Western blotting analysis revealed that the untreated preparations of the PSII-LHCII supercomplex consisted of all major PSII subunits, including the PsbO, P and Q extrinsic proteins, and also the light-harvesting components Lhcb1, 2, 4 and 5. Similar analyses of the NaCl-washed preparation showed the PsbP / PsbQ proteins to be lacking. In the Tris-treated preparation all three extrinsic proteins had been lost. Negatively stained side view averages of the supercomplexes before and after these treatments are shown in Fig 1.

In the case of the Tris-treatment no protrusions from the surface were observed and the usual dimerisation of two supercomplexes did not occur (Fig 1C). NaCl-treated supercomplexes remained as a dimer of dimers and had a centrally located protrusion from its luminal surface (Fig 1B). On the other hand untreated complexes had additional protein mass on each side of the central luminal mass (Fig 1A).

For construction of a 3D model of the supercomplex, imaging was carried out under cryo-conditions in the absence of negative stain. Around 5,000 particles were dimeric PSII assemblies deficient in one or both ends i.e. the 'tip' membrane-bound regions attributed to LHC components, and these were removed from the processing. The remaining 10,300 particles were then classified and two main populations were identified as dimeric PSII-LHCII supercomplexes ~33 nm in length, either completely lacking in density for a luminal domain (2,900), or containing the most extrinsic density observed (6,100).

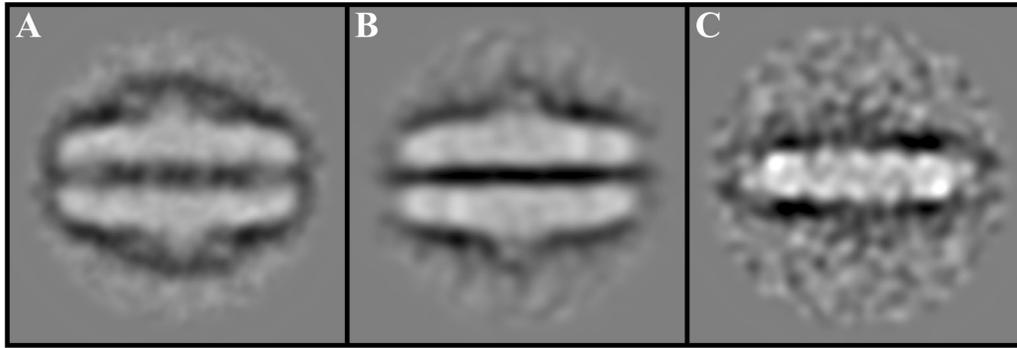


Fig 1. Negatively stained side view class averages as observed in the solubilised (A) Untreated PSII supercomplex preparation; (B) Treated with 1M NaCl; (C) Treated with 1M Tris. Observed in this last treatment were only single dimers 33 nm in length, whereas with the other two preparations, double dimers were observed.

Two 3D maps were calculated by angular reconstitution to 21 Å and 17 Å respectively. The difference map allowed for the sizing and topology of the luminal PSII density observed, including the fitting of a PsbO two-domain model, obtained by secondary structure prediction and threading (see Fig 2).

Discussion

The side view analyses of the PSII-LHCII supercomplex shown in Fig 1 confirm the earlier conclusion (Nield et al., 2000a), that the density attributed to the PsbO protein is a centrally located mass on the luminal surface when viewed from the longest side. This analysis also confirmed that the densities adjacent to the central mass are only seen when the PsbP and PsbQ proteins are present. From these experiments, we have assigned density observed in the cryo EM 17 Å map to that of the PsbO. This density has a cylindrical shape with a length of 70 Å and a diameter of 20 Å. It stretches from the luminal ends of helices 5 and 6 of the CP47 subunit across most of the luminal ends of the D1 and D2 helices, including their surface helices, to a region just to the right hand side of the transmembrane helices of CP43 (see Fig 2A)

The positioning and assignment of the transmembrane helices in Fig 2A is derived from electron-crystallography of the spinach core dimer complex (Hankamer et al., 2001) with confirmation from the recent X-ray structure (Zouni et al., 2001). The localisation of the PsbO relative to the intrinsic subunits is consistent with site-directed mutational and cross-linking studies. A remote model of the PsbO protein (Pazos et al. 2001) has been built into the density attributed to this protein (Nield et al., 2001, in preparation). This modelling suggests that the N-terminus of the PsbO is located at the CP47 side of the complex while the C-terminus extends beyond the D1 protein. Of particular significance is that our analysis and modelling indicate that the PsbO lies along the luminal surface. This is in contrast to the recent X-ray structural model derived for the core dimer complex of the cyanobacterium *Synechococcus elongatus* (Zouni et al., 2001). In this work only a portion of the PsbO protein is shown, which has a length of 30 Å protruding at a 45 degree angle to the membrane plane. Since this portion of the PsbO is located on the D1/CP43 side of the complex it would correspond to the C-terminal half of PsbO of our model. We do have a protrusion of density at about 45 degrees in our untreated supercomplex cryo-EM 3D map, but we attribute this to the presence of the PsbP and PsbQ proteins. Thus there is a difference between our work and the model derived from X-ray diffraction. We have also found this same inconsistency between single particle analysis of the core dimer complex of *S. elongatus* (see Duncan et al., 2001) and its X-ray derived structure.

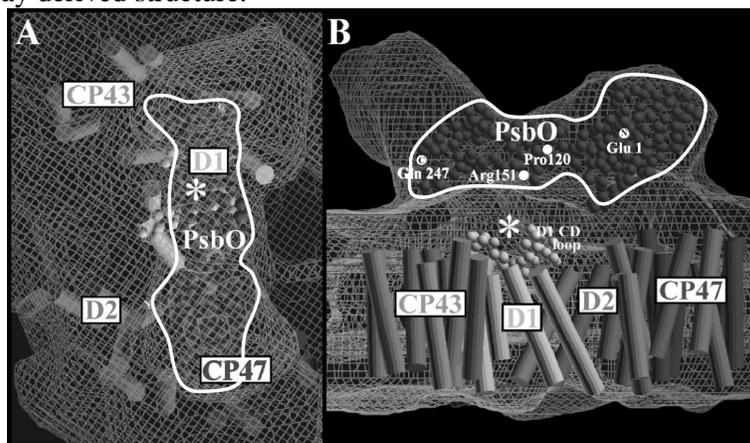


Fig 2. (A) Top view (B) Side view, of one half of the PSII dimer within a 17 Å resolved PSII supercomplex cryo-EM 3Dmap. Cylinders assigned to the underlying PSII subunit transmembrane helices are shown (see text). The remote model of PsbO is enhanced by a solid white line ~ 70 Å long x 20 Å diameter and its position was aided by comparing with the 21 Å cryo-EM 3D map of the supercomplex free of the extrinsics. The Mn cluster ~ position is shown with a *. Amino acids of interest in the PsbO remote model are as labelled.

A possible explanation for this difference is that positioning of the PsbO protein detected by X-ray diffraction is non-physiological and a consequence of the flexible nature of PsbO, involving itself in the formation of the crystal lattice. Outside of a lattice, this half of the PsbO may prefer to lie flatter across the top of the underlying membrane helices. This suggestion is supported by various studies on the isolated 33 kDa protein and in particular the modelling studies (Pazos et al. 2001), which have hinted that there is a hinge region located centrally in the PsbO remote model (around Pro 120) (see Fig 2B). This could allow for the suggested flexibility within PsbO.

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