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Biochimica et Biophysica Acta 1767 (2007) 575–582
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbabio

# Oxygen-evolving extrinsic proteins (PsbO,P,Q,R): Bioinformatic and functional analysis

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Received 30 September 2006; received in revised form 29 December 2006; accepted 25 January 2007
Available online 7 February 2007

**Abstract**

The water-splitting and oxygen-evolving (OE) reaction is carried out by a large multisubunit protein complex, Photosystem II (PSII), that has two distinct regions: a membrane intrinsic-region that includes most of the PSII subunits and a lumenal extrinsic-region that is in close association to the manganese catalytic center. The recently determined PSII 3D structures from cyanobacteria provide a considerable amount of new knowledge about the OE architecture (K.N. Ferreira, T.M. Iverson, K. Maghlaoui, J. Barber, S. Iwata, Architecture of the photosynthetic oxygen-evolving center, Science 303 (2004) 1831–1838; B. Loll, J. Kern, W. Saenger, A. Zouni, J. Biesiadka, Towards complete cofactor arrangement in the 3.0 A resolution structure of photosystem II, Nature 438 (2005) 1040–1044). Most of the intrinsic core PSII polypeptides have been well conserved through evolution from ancient cyanobacteria to modern plants, keeping the essence of PSII light driven reactions from prokaryotes to eukaryotes; but what is striking is the large number of changes that have occurred in the oxygen-evolving extrinsic proteins (OEEp) associated to PSII lumenal side. For unknown reasons plant PSII has required the "invention" of three OEEps: PsbP (23 kDa), PsbQ (16 kDa) and PsbR (10 kDa); associated to the ubiquitous OEEp PsbO (33 kDa). This set of proteins seems to be required in plants for the full activity and stability of the OE center *in vivo*, but their specific function is not clear. In this paper, bioinformatics and functional data show that the OEEps present in plants and green algae are very distinct from their prokaryotic counterparts. Moreover, clear differences are found for PsbQ from higher plants and green algae; and a relationship has been found between PsbR and the Mn cluster.


*Keywords:* Oxygen-evolving complex; Photosystem II complex; PsbO; PsbP; PsbQ; PsbR; Protein structure; Protein evolution; Bioinformatics

## 1. Introduction

The appearance of the oxygen-evolving (OE) bioreaction that liberates molecular oxygen to the atmosphere was an early event during the course of evolution and occurred in ancient cyanobacteria-like microorganisms. This fundamental reaction, powered by light energy (solar light) and present in all known oxyphototrophs, uses water ($H_2O$) as substrate and releases molecular di-oxygen ($O_2$) as by-product. The water splitting and oxygen release occurs in the catalytic center of Photosystem II (PSII), that is often termed the oxygen-evolving center (OEC) and includes a manganese-calcium cluster (4:1 Mn:Ca) as main catalytic cofactors. PSII is a large protein complex that has more than twenty different polypeptidic subunits, a major part inserted in the photosynthetic membranes as intrinsic-proteins (Ip) and another smaller set associated to the lumenal side of the complex as extrinsic-proteins (Ep). In addition to these polypeptides, PSII has many different cofactors: pigments (chlorophylls and carotenoids), metals (Mn, Ca, Fe), quinones. Recent 3D X-ray structures of cyanobacterial PSII have been obtained at 3.5 and 3.0 Å resolution [1,2], assigning most subunits and cofactors and providing a model of the OEC. However, no such high-resolution information is available for PSII of plants and green algae with the main differences being in the region corresponding to the lumenal side of the complex that includes the OE extrinsic proteins (OEEps) [3]. Here we use bioinformatics and functional data to explore the special nature of the OEEps of plants and green algae.

## 2. Methods

Isolation of PSII enriched membranes from spinach leaves was achieved as described in [4]. Calcium chloride washing of the PSII enriched membranes was conducted as described in [5]. SDS/PAGE of proteins in the membranes was performed according to the procedure described in [6]. Determination of Mn

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doi:10.1016/j.bbabio.2007.01.018

concentration in the PSII enriched membranes preparations was done using atomic absorption spectrometry. We measured the concentration of Mn (ig/ml) in the aqueous supernatant of eight PSII enriched membranes aliquots before and after $CaCl_2$ washing (see Fig. 2). To avoid any exogenous Mn (i.e. Mn not coming from PSII) controls were taken from the PSII enriched membranes before washing, not detecting any Mn in the aqueous solutions.

Protein sequence searches in public databases were done using BLAST2 [7], and HMMer [8]. Multiple sequence alignments (MSAs) were built using CLUSTAL [9] and T-coffee programs [10] and manually curated. Mature protein sequences from PsbO, PsbP and PsbQ from 10 organisms were selected and aligned. The organisms were: 5 higher plants—*Spinacia oleracea* (SPIOL), *Arabidopsis thaliana* (ARATH), *Pisum sativum* (PISSA), *Nicotiana tabacum* (NICTA), *Lycopersicon esculentum* (LYCES); 2 green algae—*Chlamydomonas reinhardtii* (CHLRE), *Bigelowiella natans* (BIGNA); and 3 cyanobacteria—*Synechocystis* sp. *PCC 6803* (SYNY3); *Nostoc* sp. *PCC 7120* (ANASP); *Thermosynechococcus elongatus BP-1* (THEEL). For the case PsbR only higher plant homologous sequences were found. Visualization and calculations on the PDB files were performed using Swiss-PDBViewer [11]. Search for structural classification was done on SCOP [12].

## 3. Results

### 3.1. Roles of the 4 extrinsic proteins associated to the plant OE complex

PsbO (33 kDa), PsbP (23 kDa), PsbQ (17 kDa), PsbR (10 kDa) are four extrinsic proteins (OEEps) associated to the lumenal side of PSII in higher plants [13]. They play an important role in maintaining oxygen evolution activity at physiological rates, but still many unclear points and questions are in the scientific arena about their specific biomolecular function and activity. PsbO is ubiquitously present in all known oxyphototrophs; however, PsbP, PsbQ and PsbR are only found in higher plants, since they are not present in known oxyphotosynthetic prokaryotes or in non-green algae (i.e. red algae). Cyanobacteria and red algae include extrinsic proteins PsbV and PsbU, but do not have the three higher plant specific OEEps, rather some of them only have some distant relatives to PsbP and PsbQ (see for details [14]). As far as we know PsbR is neither present in green algae. In this paper we address the question of why plants needed to "invent" these proteins (PsbP, PsbQ and PsbR) associated to the OE complex. To tackle this question we have done a comparative analysis of known functional data, sequences, structures and evolutionary information.

Table 1 presents a summary of the structural data and main functions attributed according to the literature to the four OEEps from plants: PsbO, P, Q and R. In terms of their structural characteristics, the table includes the PDBs of the structures of plant PsbP and PsbQ: *N. tabacum* PsbP at 1.60 Å (1V2B) [15] and *S. oleracea* PsbQ at 1.49 Å (1VYK) [16] and at 1.95 Å (1NZE) [17]; and describes the domains architecture, the SCOP structural classification and the Pfam multiple sequence alignment (MSA) and profiling of these proteins. For PsbO, the PDB is the one corresponding to cyanobacterial PSII (1S5L) [1], because the structure of this protein is at present only known as attached to PSII for prokaryotes, though it is expected to be quite similar in plants. The architecture of this protein has been analysed in detail in [18]. Finally, in the case of PsbR, there are not 3D structural data and the description of 2 domains comes from the prediction using a MSA of PsbR family and its Pfam file quoted in the table.

Table 1
Summary of the structural data and main functions attributed at present to the four OEEps from plants: PsbO, PsbP, PsbQ and PsbR

PsbO (33 kDa, 248 aa) 2 domains: 1st β-barrel cylinder, 2nd α-helix + loops head (within PSII pdb 1S5L, Pfam PF01716)
- essential for fully operational OEC since it is present in all known oxyphototrophs [13]
- stabilizes the $Mn_4Ca$ cluster providing some ligands [1,2]
- conformational sensitive to $Ca^{2+}$ and pH (active bound state = $+Ca^{2+}$ and pH ≤ 6.5) [5,30]
- seems to bind GTP/GDP, so may be involved in GTPase activity [25]

PsbP (23 kDa, 186 aa) 1 domain: 3 layer (αβα)-sandwich (pdb 1V2B, Pfam PF01789, SCOP Superfamily 55724:Mog1p/PsbP-like)
- essential for fully operational OEC *in vivo* (according to ΔpsbP in *N. tabacum*) [19]
- involved in $Ca^{2+}$ binding and supply, increasing the OEC affinity for $Ca^{2+}$ and $Cl^-$ [21]
- may bind $Mn^{2+}$ acting as a reservoir to keep/deliver Mn [22] (this binding is not in the crystal)
- may be a GTPase binding-interacting protein (according to homology with 1EQ6) [15]
- closely associated to PsbR and PsbJ [23]
- binds to PsbO head-domain by a positively charged Lys-rich region [14,26]

PsbQ (16 kDa, 149 aa) 2 domains: 1st Nt PPII, β-strands, loops, 2nd Ct 4α-helix up-down bundle (pdb 1VYK and 1NZE, Pfam PF05757, SCOP Superfamily 101113:OEEp3)
- required for PSII stability under low light conditions (according to ΔpsbQ in *A. thaliana*) [20]
- facilitates protein–protein interaction (regions: N-t polyP helix, Lys-rich face, ...) [16,27]
- binds to PsbO external cylinder-domain by a positively charged Lys-rich region [14,27]

PsbR (10 kDa, 99 aa) may be 2 domains: 1st short intrinsic, 2nd extrinsic (unknown structure, Pfam PF04725)
- closely associated to PsbP and PsbJ [23]
- associated to the OEC since it falls apart from PSII only after all 4 Mn are lost [this paper]

The functional information attributed to the OEEps from plants is also presented in Table 1 including references. Important information comes from the recent studies done on transgenic plants where PsbP [19] or PsbQ [20] proteins are not present. In *N. tabacum* lacking PsbP, PSII was hypersensitive to light and rapidly inactivated when the repair process of damaged PSII was inhibited. Moreover, the manganese cluster of PsbP-deficient leaves was markedly unstable. However, PsbQ-deficient *N. tabacum* plants did not show phenotypic alterations, suggesting that PsbQ is dispensable but PsbP is essential for full PSII function under normal growth conditions [19]. A more recent study on *A. thaliana* where both PsbQ genes (*psbQ-1* and *psbQ-2*) have been suppressed, confirms no phenotypic alteration under normal growth light but shows that PsbQ is needed for photoautotrophy under low light conditions [20]. PsbP has been implicated in $Ca^{2+}$ binding and supply, increasing the affinity of the OE center for $Ca^{2+}$ and $Cl^-$ [21]. However, more recently spinach PsbP has been reported to bind $Mn^{2+}$ and suggested to act as a reservoir capable of binding and delivering manganese to the OE center [22]. Other recent