

Minireview

Electron microscopy in structural studies of Photosystem II

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Received 13 December 2002; accepted in revised form 7 March 2003

Key words: crystal, electron microscopy, membrane protein, oxygen-evolving complex, photosynthesis, Photosystem II, single particle image analysis

Abstract

Various techniques of electron microscopy (EM) such as ultrathin sectioning, freeze-fracturing, freeze-etching, negative staining and (cryo-)electron crystallography of two-dimensional crystals have been employed, since now, to obtain much of the structural information of the Photosystem II (PS II) pigment–protein complex at both low and high resolution. This review summarizes information about the structure of this membrane complex as well as its arrangement and interactions with the antenna proteins in thylakoid membranes of higher plants and cyanobacteria obtained by means of EM. Results on subunit organization, with the emphasis on the proteins of the oxygen-evolving complex (OEC), are compared with the data obtained by X-ray crystallography of cyanobacterial PS II.

Abbreviations: 2-D – two-dimensional; 3D – three-dimensional; Chl – chlorophyll; cyt – cytochrome; DM – *n*-dodecyl-D-maltoside; EM – electron microscopy; LHC II – light-harvesting complex; OEC – oxygen-evolving complex; P680 – primary donor of Photosystem II; PAGE – polyacrylamide gel electrophoresis; PBS – phycobilisomes; PS I – Photosystem I; PS II – Photosystem II; RC – reaction center

Introduction

Oxygenic photosynthesis is a process in which plants, algae and cyanobacteria use light energy to drive the synthesis of organic compounds and produce all molecular oxygen, necessary for aerobic life on Earth (see Whitmarsh and Govindjee 1999). The light-harvesting and energy-transducing functions of oxygenic photosynthesis are localized in specialized photosynthetic membranes, thylakoids, and carried out by several types of protein complexes embedded in the membrane: Photosystem II (PS II), Photosystem I (PS I), cytochrome *b*₆/*f* (cyt *b*₆/*f*) and ATP synthase. Structural and functional similarities of the complexes in the thylakoids between prokaryotes (cyanobacteria) and eukaryotic cell (algae and higher plants) are sugges-

ted by the endosymbiont theory, which postulates an intracellular symbiosis of a photoautotrophic organism with a heterotrophic eukaryotic cell (Gray 1992; McFadden 1999).

This paper reviews recent information about the progress and present situation in studies on the structural organization of PS II in photosynthetic thylakoid membranes of higher plants and cyanobacteria. Various techniques of electron microscopy (EM) have been employed during recent years to improve our current knowledge about PS II structure at both low and high resolution. Low-resolution structural data, obtained from ultrathin sectioning and freeze-fracture and freeze-etch EM studies are outlined first. Such studies have yielded information on the overall organization of thylakoid membranes and the location of PS

II and its antenna system and the other components of thylakoid membrane. Higher resolution data have been obtained by either single particle image analysis of detergent-solubilized PS II particles or analysis of two-dimensional (2-D) crystals and are described later. These methods together with biochemical analyses have provided substantial information on the subunit organization, oligomeric state and interaction of PS II subunits with its antenna system. A 3-D model of the PS II complex has been recently resolved by X-ray crystallography. These results, with emphasis on proteins of the oxygen-evolving complex (OEC), are compared with those obtained by EM techniques.

Photosystem II

PS II is a multisubunit pigment–protein complex embedded in the thylakoid membranes of higher plants, algae and cyanobacteria (Hansson and Wydrzynski 1990; Ghanotakis and Yocum 1990; Barber 1998). It performs a series of photochemical reactions, which result in the reduction of plastoquinone, the oxidation of water, and the formation of a transmembrane pH gradient. The primary electron donor of PS II, P680⁺, is the strongest oxidant generated in biological systems. Such high potential (+1.17 V) enables PS II to extract electrons from water to produce molecular oxygen.

More than 25 subunits (coded by genes *psbA*–*psbZ*) have been identified in association with PS II (Ghanotakis and Yocum 1985; Ikeuchi 1992; Barber et al. 1997). The central part of the complex is formed by a reaction center (PS II-RC) composed of the D1 and D2 proteins associated with α and β -subunits of cytochrome *b*₅₅₉ (cyt *b*₅₅₉) and PsbI protein (Nanba and Satoh 1987). The reaction center is surrounded by chlorophyll *a* (Chl *a*)-binding inner antenna proteins CP47 and CP43 (Bricker 1990; Barber et al. 2000; Bricker and Frankel 2002). Three extrinsic proteins with molecular masses of 33, 23 and 16 kDa are attached to the luminal surface of the complex forming the oxygen-evolving complex (OEC) that maintains an optimal environment for water oxidation (Murata and Miyao 1985; Seidler 1996). Several low-molecular weight proteins with unknown functions are also associated with the whole PS II core complex (Barber et al. 1997; Hankamer et al. 2001a).

Major differences between cyanobacteria and higher plants are found in the outer PS II antenna system. The antenna system of cyanobacteria is

formed by water-soluble phycobilisomes (e.g., Glazer 1982; Mörschel 1991; MacColl 1998). These supramolecular complexes are composed of phycobiliproteins with covalently attached open-chain tetrapyrroles (phycobilins). The antenna system of higher plants and green algae consists of membrane-bound Chl *a/b*-binding proteins coded by *lhcb1*–*6* genes (Jansson 1994; Paulsen 1995; Green and Durnford 1996). The Lhcb1 and Lhcb2 proteins form a major heterotrimeric light-harvesting complex of PS II (LHC II) whose structure was determined by electron crystallography (Kühlbrandt et al. 1994). The remaining minor Lhcb proteins [CP29 (Lhcb4), CP26 (Lhcb5), and CP24 (Lhcb6)] are suggested to be monomeric and function as linker proteins between the trimeric LHC II and PS II core complex (Green et al. 1991; Sandonà et al. 1998).

Organization of thylakoid membranes

Electron micrographs of ultrathin sections of chloroplasts revealed the overall morphology of thylakoid membranes of higher plants. Thylakoid membranes are differentiated into stacked (appressed) and unstacked (unappressed) regions. The unstacked regions are termed stroma lamellae whereas the stacked regions form grana membranes. Unstacked regions are termed stroma lamellae whereas stacked regions form grana membranes. Grana membranes are approximately circular with a diameter of about 500 nm and are in close contact with two thylakoid vesicles, as in green algae, or up to as many as 20, as in higher plants. A 3-D reconstruction from serial sections through the entire chloroplast led to the proposal of several models of spatial architecture of thylakoid membranes (Mustardy 1996; Arvidsson and Sundby 1999). Immunogold-labeling experiments with antibodies against various components of the membrane revealed a marked lateral distribution of main complexes (Vallon et al. 1985, 1986). While PS I and ATP synthase are exclusively located in stroma membranes, PS II and LHC II are predominantly found in grana membranes (see Olive and Vallon 1991; Olive and Wollman 1998). These results had been previously suggested by fractionating analysis (Andersson and Akerlund 1978; Andersson and Anderson 1980) and confirmed by freeze-fracture/etching techniques (Olive and Vallon 1991; Staehelin and van der Staay 1996; Olive and Wollman 1998), negatively stained

preparations (Boekema et al. 1994; Hankamer et al. 1997a) and spectroscopically (Vácha et al. 2000).

Ultrathin sections of cyanobacteria revealed that their thylakoid membranes lack both stacked and unstacked domains (see Gannt 1994). The phycobilisomes (PBS) are attached to the stromal surface of the membrane and are most commonly arranged alternately between two adjacent membranes (Olive et al. 1997). Contrary to the lateral heterogeneity found in higher plants, immuno-labeling studies on cyanobacterial ultrathin sections revealed a relatively homogenous distribution of the complexes within the membrane (Mustardy et al. 1992), although some asymmetry has been detected (Sherman et al. 1994; Marquardt et al. 2000).

Organization of Photosystem II within the membrane

Higher plants

Freeze-fracture and freeze-etching electron microscopy techniques are excellent tools for visualizing *in vivo* organization and distribution of integral membrane protein complexes within the thylakoid bilayer. In these studies, protein complexes are visualized as 'particles' and are named according to the planes with which they are associated (Figure 1). Although these methods cannot be used to describe the protein composition of the particles, they provide structural information on the localization, morphology, heterogeneity, dimensions, and shapes of PS II and its antenna components at a resolution of about 5 nm (Staehelin and Arntzen 1983; Staehelin and deWitt 1984; Olive and Vallon 1991; Staehelin and van der Staay 1996; Olive and Wollman 1998).

Comparative structural and biochemical investigations have been used to determine the identity of the particles including such as different illumination conditions (Armond et al. 1977; Olive et al. 1981), PS II-deficient mutants (Miller and Cushman 1979; Wollman et al. 1980; Lacambra et al. 1984; Olive et al. 1992) and reconstitution of protein complexes into liposomes (Sprague et al. 1985). These results indicated that EFs (*endoplasmic fracture face of luminal leaflet of stacked membrane*, Figure 1) and EFu (*endoplasmic fracture face of luminal leaflet of unstacked membrane*) particles correspond to PS II complexes. The significant difference between EFs and EFu planes was in their respective particle sizes.

The EFs particles range from 11–16 nm across while EFu particles are only 8 to 11 nm across. Based on these studies it has been suggested that the EFs particle corresponds to a dimeric PS II complex and variations in size are caused by a lack of one or several associated minor antenna proteins (CP29, CP26, CP24). The composition of EFu particles is less clear but the results indicate it to be PS II in its monomeric form. Monomeric PS II complexes have been isolated from the stroma region (Santini et al. 1994; Bassi et al. 1995; Dekker et al. 2002) and showed different structural and functional properties from the granal counterparts (Melis 1991; Jansson et al. 1997; Mamedov et al. 2000). These results are in good agreement with a 'PS II-repair cycle' which involves a monomerization and transfer of the damaged PS II complexes from grana to stroma region, partial disassembly, replacement of newly synthetized D1 protein and finally return of the rebuilt complex to grana membranes (Barbato et al. 1992; Barber and Andersson 1992; Aro et al. 1993; Kruse 2001; Baena-Gonzales and Aro 2002).

Freeze-etching of endoplasmic (luminal) surface of stacked thylakoid membranes (ESs) revealed particles with a tetrameric appearance (Miller et al. 1976; Simpson 1978; Miller and Cushman 1979). The ESs particles closely correspond to EF particles and also exhibit similar lattice properties when arranged in regular arrays (Figure 2[1c, d]). Sequential removal of the extrinsic proteins (16, 23, and 33 kDa) resulted in a particle height reduction (from 8.2 nm to 7.8, 7.4 and 6.1 nm, respectively) and change in particle substructure appearance (Seibert et al. 1987). Reconstitution of the depleted membrane fragments with the OEC proteins led to a rebinding of extrinsic proteins and reappearance of the tetrameric ESs particles (Simpson and Andersson 1986). It has been concluded that ESs tetrameric particles correspond to the dimeric PS II complex and the protruding elements are formed by two copies of the OEC subunits (Staehelin 1976; Dunahay et al. 1984).

Analysis of mutants lacking LhcB proteins (Miller et al. 1976; Simpson 1979) and plants grown under intermittent light (Armond et al. 1977) indicated that 8 nm particles associated with the PFs plane (*protoplasmic fracture face of stromal leaflet of stacked membrane*) correspond to PS II antenna proteins. These studies showed a substantial reduction of PFs particles in membranes lacking LhcB proteins, compared to wild-type thylakoids, and also a decrease of EFs particle size indicating closely asso-

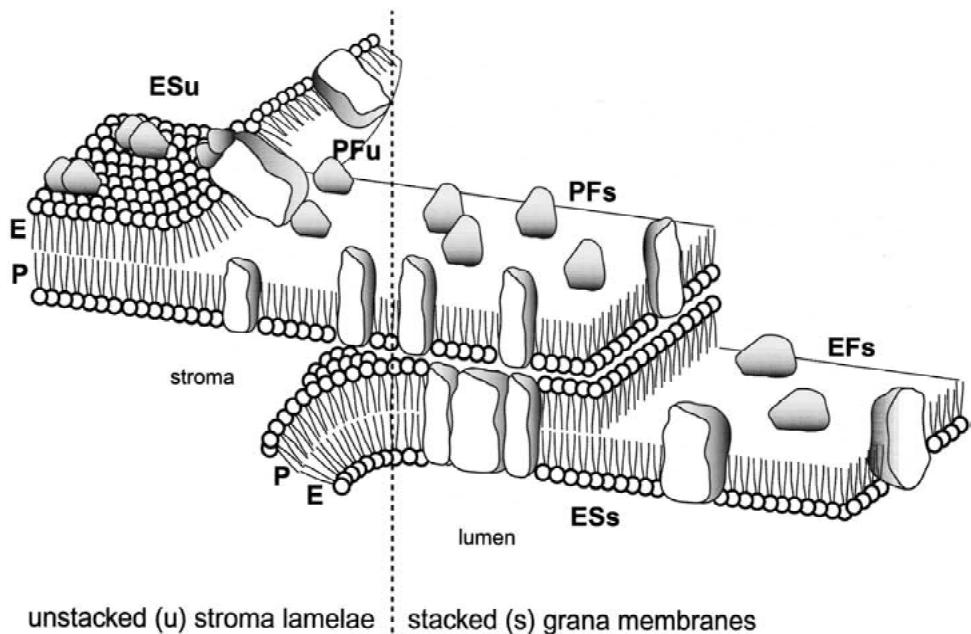


Figure 1. Schematic diagram illustrating freeze-fracture/etching of thylakoid membrane of higher plants. In freeze-fracture a sample is rapidly frozen at temperatures below -100°C to produce a planes of fractures through the specimen. Fracture planes tend to pass along the central hydrophobic plane of the lipid bilayer to produce two complementary fractured faces. Since the integral protein complexes that span the membrane bilayer are not splitted during the fracturing process, they are seen as 'particles' that rise above a smooth surface. In freeze-etching studies, fracture planes also often pass along lines of weakness such as the interface between cytoplasm and membrane, so that the outer and inner membrane surfaces can be viewed. The fractured specimen is left to evaporate water under vacuum and exposes the membrane surface and allows the visualization of extrinsic components in their native state. Using freeze-fracture/etching techniques thylakoid membrane is splitted to Endoplasmic (E) and Protoplasmic plane (P) that corresponds to luminal and stromal leaflet of the membrane, respectively. We can distinguish Fracture plane (F) and original Surface (S) referred to endoplasmic surface ES, endoplasmic fracture EF, protoplasmic surface PS, and protoplasmic fracture PF (Branton et al. 1975). In addition, the stacked (s) and unstacked (u) membrane regions are present in chloroplast, giving rise to four types of freeze-fracture planes: EFs, PFs, EFu and PFu (Staehelin 1976).

ciated minor antenna proteins (Olive et al. 1981). In addition, the crystal structure of LHC II (Kühlbrandt et al. 1994) confirmed the assumption that 8 nm PF particles correspond to the trimeric LHC II.

Since EF and PF planes constitute two artificially produced views of originally only one membrane (see Figure 1), it can be assumed that EFs and PFs are complementary fracture planes. This assumption was strengthened by the analysis of EFs and PFs particles organized into 2-D arrays. Simpson (1978) indicated that EFs arrays have a lattice with unit cell dimensions of 24.7×17.5 nm. PFs arrays contained 8 nm particles in double rows separated by deep grooves with a spacing of 24 nm (Figure 2[1c]). Careful examination of both fracture planes showed that each EFs particle (PS II dimer) is laterally associated with PFs particles (LHC II) in a ratio of either 1:2 (Staehelin 1975; Tsvetkova et al. 1994) or 1:3 (Simpson 1978). Such associations of dimeric PS II complex with various amounts of Lhcb subunits have been investigated

by image analysis of negatively stained preparations and the results are mentioned in the section 'Negative staining of PS II particles'.

Cyanobacteria

Thylakoid membranes of cyanobacteria are densely populated with protein complexes and, in addition to the components of the photosynthetic electron transport chain contain also the components of the respiratory chain. Both electron transport pathways can intersect which has been observed in mutants (Vermaas 1994). Freeze-fracture studies of cyanobacterial thylakoids revealed particles with a dimension of 20×10 nm associated with EF (endoplasmic fracture face of luminal leaflet of the membrane) plane (Neushul 1971; Mörschel and Mühlthaler 1983). The EF particles were separated into two parts (10×10 nm) and were mostly aligned in long parallel rows (Mörschel and Schatz 1987). Comparative structural studies revealed that EF particles correspond to PS

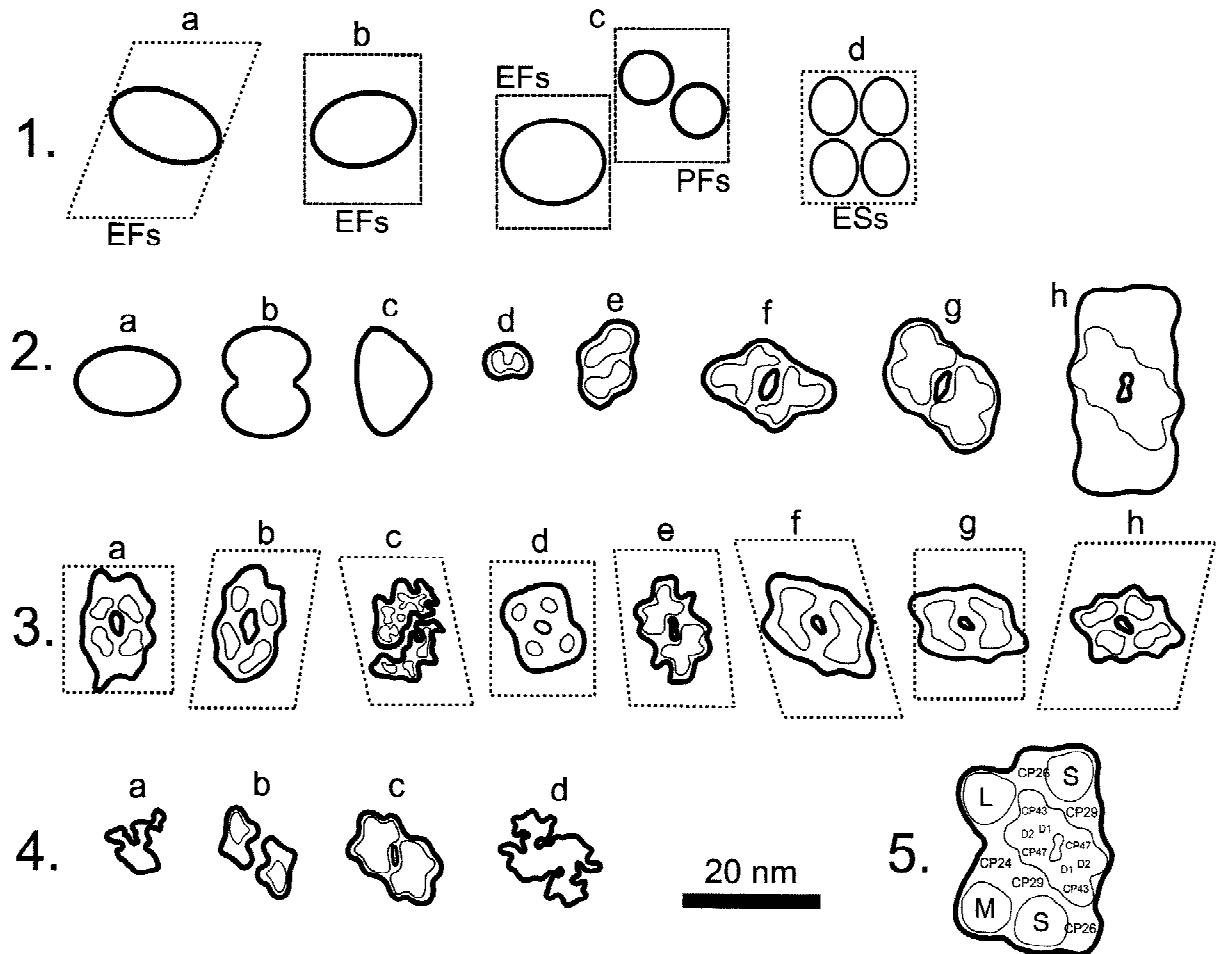


Figure 2. Schematic representations of PS II structures as reported by various authors.

1. Projection contours of the particles associated with EFs, PFs and ESs planes obtained by analysis of EM images of 2-D particle arrays from freeze-fracture and freeze-etching experiments: (a) 2-D array of EFs particles from spinach (Simpson 1978). (b) 2-D array of EFs particles from *Arabidopsis* (Tsvetkova et al. 1995). (c) 2-D arrays of EFs and PFs particles from two complementary membrane fracture planes (Staehelin 1975). (d) 2-D array of tetrameric ESs particles obtained by freeze-etching method (Seibert et al. 1987).
2. Top-view projection contours of negatively stained PS II particles obtained by EM and single particle analysis: (a) and (b) Cyanobacterial monomer (a) and dimer (b) (Rögner et al. 1987). (c) Spinach monomer (Haag et al. 1990). (d) Reaction center monomer (Tsiotis et al. 1999). (e) Spinach monomer (Hasler et al. 1997). (f) Spinach dimer (Boekema et al. 1995). (g) Dimeric CP47-RC subcomplex (Eijkelhoff et al. 1997). (h) Spinach PS II-LHC II supercomplex (Boekema et al. 1995).
3. Projection contours of PS II complexes derived from crystallographic analysis of EM images of 2-D crystals as reported by the authors: (a) Spinach monomer in 2-D array (Holzenburg et al. 1993). (b) Barley monomer in 2-D array (Stoylova et al. 2000). (c) Barley monomer in 2-D array (Ford et al. 1997). (d) Maize dimer in 2-D array (Santini et al. 1994). (e) Barley dimer in 2-D array (Marr et al. 1996b). (f) and (g) Large-spaced (f) and small-spaced (g) 2-D arrays from spinach (Boekema et al. 2000a). (h) *Arabidopsis* dimer in 2-D array (Yakushevska et al. 2001). The unit cells calculated from the 2-D arrays are indicated by dotted line (1a-d, 3a-h).
4. Projection contours of PS II complexes derived from crystallographic analysis of EM images of 2-D crystals from reconstituted PS II particles as reported by the authors: (a) Monomeric CP47-RC subcomplex (Rhee et al. 1998). (b) Monomeric PS II core complex from spinach (Tsiotis et al. 1996). (c) and (d) Dimeric PS II core complex from spinach, (c) Morris et al. (1997), (d) Hankamer et al. (1999).
5. Model of supramolecular organization of spinach PS II-LHC II supercomplex including a dimeric PS II core complex (CP47, CP43, D2, D1) connected with two strongly bound (S) LHC II trimers (cf. Figure 2[2h]). The two additional LHC II trimers bound at moderately (M) and loosely (L) positions are attached to left side of the PS II-LHC II supercomplex. The location of monomeric LhcB4 (CP29), LhcB5 (CP26) and LhcB6 (CP24) proteins are indicated (redrawn from Boekema et al. 1999a). The scale bar measures 20 nm.

II complex. The PS II-deficient mutant lacked EF particles in comparison with the wild-type thylakoids (Nillson et al. 1992). The absence of EF particles was also observed in heterocysts which lacked any PS II activity (Giddings and Staehelin 1979).

Freeze-etching of cyanobacterial thylakoids showed phycobilisomes to be arranged in parallel rows with a spacing of 45 nm and periodicity of 10 nm (Giddings et al. 1983). The spacing of the PBS rows closely corresponds to the distances between the EF particle rows and can decrease to 16 nm in the absence of assembled PBS structures (Olive et al. 1997).

A model for organization of the PS II complex in cyanobacterial thylakoids has been proposed (Giddings et al. 1983; Bald et al. 1996; Mullineaux 1999). One phycobilisome is attached to the dimeric PS II complex and these PS II-PBS supercomplexes are organized into tightly arranged parallel rows, with PS I and cyt *b*₆/*f* in the spaces between the rows. As the width of PBS is about 15 nm and the width of the PS II dimer is only 11 nm the long axis of each phycobilisome is not parallel to the long axis of the dimer but it is tilted to achieve a contact between neighboring PS II particles (Bald et al. 1996).

Breakdown fragments of these rows have been isolated from thylakoids of *Synechococcus elongatus*. Electron micrographs of negatively stained fragments revealed that they appear as 'double dimers' with an estimated molecular mass of 1000 kDa (Kühl et al. 1999). Fitting the projection map of cyanobacterial PS II dimer (Boekema et al. 1995; Kühl et al. 1999) into one half of the double dimer revealed that the dimer has an overlap of about 0.6 nm in the center and 2.2 nm at the periphery. This has been explained by the detergent boundary layer that surrounds the isolated single dimers, but it is missing in the hydrophobic contact area of the double dimers. A repeating distance of PS II dimers in the row was calculated to be 11.7 nm and an angle between the long axis of the dimer and the rows about 73 °C (Kühl et al. 1999).

Formation of the PS II rows in the cyanobacterial thylakoids probably results in an effective separation of PS I and PS II membrane domains (Trissl and Wilhelm 1993). Since PS I is faster trap than PS II this separation may help efficiently distribute the excitation energy between these two photosystems. Generally, the ability to maintain efficient light-energy conversion is related to the distribution of absorbed energy between two photosystems, called 'state transition'. This short-term adaptation process is a regulatory mechanism that allows a preferential excitation

of PS I or PS II by selective enhancement of excitation energy transfer to the less active photosystem (see Fujita et al. 1994; Rögner et al. 1996). The mechanism of this adaptation is still conjectural with respect to lateral redistribution of the complexes within the membrane (Olive et al. 1997).

Negative staining of PS II particles

Single particle image analysis has been used to study the structure of a wide range of biological molecules and macromolecular complexes (see Ruprecht and Nield 2001). The samples are placed on an electron microscope grid and either imaged in heavy atom salts (such as uranyl acetate) or in vitreous ice (cryo-electron microscopy). The particles from EM images are classified according to the orientation of the particle on the grid, and members of each class are then rotationally and translationally aligned. Recently, labeling of His-tagged proteins with a Ni²⁺-NTA gold cluster and single particle analysis have been shown to be a novel approach for localizing individual protein subunit within the multisubunit complex (Hainfeld et al. 1999; Büchel et al. 2001).

Negative staining and single particle analysis have been used to analyze a number of solubilized PS II complexes (see Boekema et al. 1994; Nicholson et al. 1996; Tsiotis et al. 1996a; Hankamer et al. 1997a). The first work concerning PS II structure has been published by Rögner et al. (1987). The complexes with an estimated molecular mass of 300 and 500 kDa (Figure 2[2a, b]) indicated monomeric and dimeric PS II complex, respectively (Irrgang et al. 1988). Dimensions of 18 × 15 nm for dimeric particle and 16 × 11 nm for monomeric particle were reported in their top-view projections (Dekker et al. 1988). The side-view projection revealed a small protrusion on the luminal side of the complexes that has been assigned to the 33 kDa extrinsic subunit (Haag et al. 1990).

The comparative projection maps of both cyanobacterial (*S. elongatus*) and a spinach PS II core complex have been demonstrated by Boekema et al. (1995). Both complexes had similar protein and pigment composition consisting of CP47, CP43, D1, D2, cyt *b*₅₅₉, the 33 kDa subunit, together with about 38 Chla molecules (Hankamer et al. 1997b). Gel filtration experiments showed that both PS II core complexes had a molecular mass of about 450 kDa. Dimensions of these particles were calculated to be 20.6 × 13.1 nm in their top-view projections (Figure 2[2f]). In overall

topology the densities on both sides of the complex were arranged in a similar way, with two-fold rotational symmetry around the center, and thus, a dimeric organization of the complexes has been proposed. This result has been confirmed by the analysis of electron micrographs obtained with PS II core complexes having a molecular mass of 240 kDa (Fig. 2[2e]). These particles had dimensions of 14×9.5 nm were supposed to be a monomeric PS II complex. By comparing these two oligomeric states it can be seen that the projection map of the monomeric complex corresponds well with each half of the PS II core dimer (Boekema et al. 1995).

Monomeric PS II complex has been also isolated from spinach and *Synechococcus* sp. OD24 (Hasler et al. 1997). The molecular mass of the complexes was determined by scanning transmission electron microscopy (STEM) to be 281 kDa for spinach and 313 kDa for cyanobacterial PS II complex (Figure 2[2e]). Single particle analysis revealed that PS II complexes were comparable in size and shape (approx. 14×10 nm). The averaged projection exhibited four distinct protein densities in a pseudo-twofold symmetry. Two of these four densities have been attributed to contain reaction center proteins (D1/D2) in accordance with data obtained by STEM analysis of isolated PS II reaction centers particles (Figure 2[2d]) from spinach (Tsiotis et al. 1999).

Although monomeric PS II core complexes can be isolated from both cyanobacteria and higher plants, many results have showed that the dimeric form is present *in vivo* in thylakoid membranes. Isolated dimeric PS II core complexes are more active in oxygen evolution and have a higher level of the 33 kDa extrinsic proteins. They are also characterized by a higher pigment content and have fewer D1/D2 breakdown fragments than its monomeric counterpart (Hankamer et al. 1997b). Strong evidence against a possible monomeric organization of PS II complex in thylakoid membrane is that monomeric PS II particles have never been isolated with any of the Lhcb subunits. Non-denaturing polyacrylamide gel electrophoresis (PAGE) confirmed that grana membranes are consisted predominantly of dimers, while stromal parts are enriched in PS II monomers (Dainese and Bassi 1991; Peter and Thornber 1991; Bassi et al. 1995).

Nevertheless, artificial dimeric aggregations of PS II core complexes and CP47-RC subcomplexes have been induced by gel filtration chromatography (Eijckelhoff et al. 1997). From a comparison between

projections of PS II core complexes and CP47-RC subcomplexes the position of the CP43 subunit at the tip of the complex has been determined (Figure 2[2f and 2g]).

Mild detergent treatment of grana membranes prior to sucrose density gradient centrifugation resulted in the isolation of the PS II-LHC II supercomplex (Boekema et al. 1995). Electron microscopy and single particle analysis showed that the supercomplex has a two-fold rotational symmetry with a dimension of 30.2×15.7 nm in its top-view projection (Figure 2[2h]). The superimposition of the dimeric PS II core complex (Boekema et al. 1995) upon the projection map of the supercomplex indicated that a central part is occupied by a dimeric PS II core complex and each region on both sides has been suggested to contain a trimeric LHC II and a single copy of CP29 and CP26 proteins. To relate the top-view projection of the supercomplex with the 2-D arrays of ESs particles (Figure 2[1d]) observed by freeze-etching studies of grana membranes (Seibert et al. 1987), a similar lattice (21×19 nm) within the supercomplex has been constructed (Hankamer et al. 1997b). The dimensions of both lattices correlated well and the authors suggested that crystalline ESs arrays observed in thylakoid membrane are composed of the PS II-LHC II supercomplexes (Hankamer et al. 1997b).

Recently, a three-dimensional (3-D) structure of the supercomplex from spinach (Nield et al. 2000b) and *Chlamydomonas reinhardtii* (Nield et al. 2000a) has been presented. The structural similarities between spinach and algal supercomplexes led the authors to conclude that the PS II-LHC II supercomplex forms a basic unit in Chl_b-containing organisms.

The associations of additional LHC II trimers to the PS II-LHC II supercomplex have been studied by an α -DM treatment of the PS II-enriched membranes and single particle analysis (Boekema et al. 1998b, 1999a, b; Yakushevska et al. 2001). These studies have shown that LHC II trimers can be attached in three different types of positions, referred to as strongly (S), moderately (M) or loosely (L) bound LHC II trimers (see Figure 2[5]). Two S-type trimers have been previously proposed to flank the dimeric PS II complex and together form the PS II-LHC II supercomplex (Figure 2[2h]). The latter two types (M and L) were associated on both sides of the supercomplex. The M-type trimer has been placed to connect the supercomplex through the S-type trimer and CP29, whereas the L-type trimer was directly attached to the dimeric PS II core (Boekema et al. 1999a). In contrast to spin-

ach, the M-type trimers from *Arabidopsis* have been shown to bind dimeric PS II in the absence of S-type trimer and no binding of the L-type trimers have been detected (Yakushevska et al. 2001). A possible route of the biogenesis of complete PS II complex has been suggested: in the first instance CP29 binds to the dimeric PS II core complex, followed by trimeric LHC II and then CP26 and CP24 (Boekema et al. 1999a).

2-D Crystallography of PS II

Electron crystallography is a powerful technique for the structural determination of membrane proteins at high resolution and has been successfully used for solving atomic structures of bacteriorhodopsin (Henderson et al. 1990) and plant light-harvesting complex II (LHC II) (Kuhlbrandt et al. 1994). The resolution of the data is limited to the size and quality of the two-dimensional (2-D) crystals and their preparation is one of the major bottlenecks in the crystallographic studies. Many reports concerning 2-D crystallography of PS II complex have been published and the results are summarized in Table 1. It is interesting to note that almost all crystallization attempts utilized only higher plant thylakoid membranes (pea, spinach, barley, maize) as a starting material. Generally, three approaches have been employed for structural analysis of PS II 2-D crystals by electron microscopy: (i) analysis of the ordered 2-D arrays (PS II crystalline arrays) in the native thylakoid membrane, (ii) *in situ* crystallization procedures including a detergent-induced delipidation of PS II-enriched membranes, and (iii) reconstitution of isolated PS II (sub)complexes with thylakoid lipids.

Native 2-D crystalline arrays of PS II

Several protocols have been developed to isolate membrane fragments enriched in PS II (Berthold et al. 1981; Kuwabara and Murata 1982; Yamamoto et al. 1982; van der Staay and Staehelin 1994). These preparations are highly active purified grana membranes, composed of paired, appressed membrane fragments with their luminal side exposed (Dunahay et al. 1984). Recently, similar PS II-enriched membrane fragments have been prepared by solubilization of thylakoid membranes with α -dodecyl-maltoside (α -DM) (van Roon et al. 2000).

Electron micrographs of grana membranes revealed that PS II particles are more or less randomly

distributed within the grana (e.g., Kitmitto et al. 1999) or organized in 2-D arrays (Staehelin 1976; Simpson 1979; Dunahay et al. 1984; Bassi et al. 1989; Yakushevska et al. 2001). The particle arrays are occasionally seen in chloroplasts isolated from wild-type plants whereas a tendency to form regular arrays is increased in various types of mutant plants. Induction of array formation can be also triggered after isolation by changing the composition of the suspending medium (Simpson 1979; Tsvetkova et al. 1995). The factors leading to their formation are still poorly understood but growth conditions (Stoylova et al. 2000) or lipid compounds (Tsvetkova et al. 1995) are of importance.

Since negatively stained 2-D arrays of PS II membrane fragments provide a higher resolution (2 nm as compared to 5 nm for 'freeze' techniques) more structural details about the organization of PS II and LHC II complexes have been obtained. The EFs (ESs) particle arrays clearly correspond to stain-excluding regions within the PS II particle 2-D arrays in terms of particle size and shape and could be definitely known to contain the PS II complexes. The lattice dimensions varied slightly among various reports (see Table 1) but single unit cells showed certain similarities. The unit cell contains two predominant stain-excluding areas with predicted two-fold rotation symmetry indicating a dimeric PS II. Relatively large stain density areas between the PS II particles have been suggested to include the light-harvesting proteins in accordance with the absence of large hydrophilic loops that causes low protein density regions on 2-D crystals of LHC II (Lyon and Unwin 1988).

Detailed analysis of two types of PS II 2-D arrays has been reported by Boekema et al. (2000a). A dimeric PS II complex, associated with two or three additional trimers of LHC II, has been fitted into the small-spaced and large-spaced lattice, respectively (Figure 2[3f, g]). The ratios of 1:2 and 1:3 between dimeric PS II and LHC II trimers observed in negatively-stained preparations (Boekema et al. 2000a) are in good agreement with the analysis of EFs and ESs particle arrays obtained by freeze-fracture/etching techniques (Staehelin 1975; Simpson 1978). It has been shown that the PS II-LHC II supercomplex can be fitted into both lattices. The small-spaced lattice contained tightly arranged PS II-LHC II supercomplexes while the large-spaced lattice accommodated extra mass which could be occupied by an additional trimeric LHC II together with CP24 protein (Boekema et al. 1998b).

Table 1. Electron crystallographic data of the 2-D crystals of PS II and their interpretations to the structural properties of PS II. The table provides the data on plant material, unit cell parameters and oligomeric state of PS II according to authors. The unit cell parameters of (i) *native 2-D crystalline PS II arrays* are comparable in their size and correspond to different organization of the dimeric PS II–LHC II supercomplexes within the lattices. The unit cell parameters of (ii) *detergent-induced PS II 2-D crystals* differ substantially among the reports. Since the structural features of the unit cells are comparable (see Figure 2) the variations in their sizes are probably caused by an unequal number of attached antenna proteins. (iii) *2-D crystals from reconstituted particles* have uniform unit cell parameters and it was proved that they consist of PS II dimers. Such unit cells can be superimposed over all the other preparations reported in this table suggesting the dimeric nature of PS II complexes in these preparations

Plant material	Symmetry, unit cell parameters (nm) and angle	Notation in Figure 2	Intepretation of the unit cell according to the authors	Reference
<i>Native 2-D crystalline PS II arrays</i>				
Spinach	<i>p</i> 2 27.3 × 18.3, 75°	3f	PS II dimer + 3 × LHC II trimer	
	<i>p</i> 2 23.0 × 16.9, 90°	3g	PS II dimer + 2 × LHC II trimer	Boekema et al. (2000a)
<i>Arabidopsis</i>	<i>p</i> 2 25.6 × 21.4, 77°	3h	PS II dimer + 4 × LHC II trimer	Yakushevska et al. (2001)
<i>Detergent-induced PS II 2-D crystals</i>				
Spinach	<i>p</i> 1 18.9 × 16.8, 89°	3a	PS II monomer (890 kDa)	Holzenburg et al. (1993)
Barley	<i>p</i> 1 24.3 × 16.2, 81°	3a	PS II monomer + monomeric LHC subunits	Stoylova et al. (2000)
(far-red light)	<i>p</i> 1 21.5 × 17.5, 87°			
Barley	<i>p</i> 1 23.0 × 15.5, 97°	3c	PS II monomer	Ford et al. (2002)
Maize	<i>p</i> 2 26.7 × 17.8, 90°	–	2 × PS II dimer (2000 kDa)	Bassi et al. (1989)
Maize	<i>p</i> 2 20.5 × 16.1, 90°	3d	PS II dimer (672 kDa)	Santini et al. (1994)
Barley	<i>p</i> 2 24.1 × 16.1, 84°	3e	PS II dimer (740 kDa)	Marr et al. (1996b)
Spinach	<i>p</i> 2 16.7 × 12.0, 75°			
	<i>p</i> 2 16.4 × 15.1, 69°	–	PS II dimer	Lyon (1998)
	<i>p</i> 2 15.6 × 10.8, 74°			
<i>2-D crystals from reconstituted particles</i>				
Spinach	<i>p</i> 22 ₁ 2 ₁ 16.7 × 15.3, 90°		4 × CP47-RC subcomplex	Nakazato et al. (1996)
Spinach	<i>p</i> 22 ₁ 2 ₁ 16.8 × 15.5, 90°	4a	(each 150 kDa)	Rhee et al. (1998)
Spinach	<i>p</i> 1 16.2 × 13.7, 142°	4b	PS II monomer (600 kDa)	Tsiotis et al. (1996)
Spinach	<i>p</i> 2 ₁ 2 ₁ 2 ₁ 17.3 × 11.7, 70°	4c	PS II dimer (430 kDa)	Morris et al. (1997)
Spinach	<i>p</i> 2 ₁ 2 ₁ 2 ₁ 17.5 × 12.7, 107°	4d	PS II dimer (430 kDa)	Hankamer et al. (1999)
<i>Synechococcus</i>	<i>p</i> 22 ₁ 2 ₁ 33.3 × 12.1, 90°	–	2 × PS II dimer	daFonseca et al. (2002)

Recently, PS II 2-D arrays in *Arabidopsis* have been reported by Yakushevska et al. (2001). Parameters of a unit cell ($\alpha = 25.6$, $\beta = 21.4$ nm, $\gamma = 77^\circ$) indicated the largest PS II lattice that had been ever observed (Figure 2[3h]). The PS II–LHC II supercomplex with two additional trimeric LHC II's has been fitted into the lattice suggesting the presence of four LHC II trimers attached to the dimeric PS II complex (Yakushevska et al. 2001).

It should be noted that the positions of LHC II trimers within the lattice did not correspond to boundaries of the unit cells and are overlapping. By a closer inspection of the PS II 2-D arrays (Figure 2[3f–h]) one can suppose that the dimeric PS II–LHC II supercomplex forms the basic motif of the arrays and the small changes in arrangement might be caused by 'gliding' the supercomplexes along their long axis. However, the exact nature of the material in low-density areas is in debate and requires further examination.

Detergent-induced 2-D crystals of PS II

Formation of PS II 2-D crystals can be induced by a detergent treatment of thylakoid membranes. The detergent preferentially solubilizes the non-stacked region of the membrane leaving grana membranes intact. Furthermore, the formation of crystals involves dissociation of LHC II from PS II to separate areas, specific associations between the PS II complexes and gradual depletion of lipid molecules.

Several different types of PS II crystals have been produced using a variety of detergents such as Triton (Bassi et al. 1989; Lyon et al. 1993; Marr et al. 1996b), dodecyl-maltoside (Holzenburg et al. 1993; Santini et al. 1994; Ford et al. 1995; Lyon 1998) or tetradecyl-maltoside (Lyon 1998). Although each crystal type has been characterized by different unit cell parameters (see Table 1) a comparison of contour maps in Figure 2[3a–e] shows that projection maps have very similar structural features. A central low-density area is flanked by two predominant regions of protein density, which appear to be related by a two-fold rotational symmetry axis. The two apparent protein densities were similar in all preparations and clearly correspond to a PS II dimer. It appears that unit cells vary only in the different arrangements of the dimers suggesting specific interactions between the proteins within PS II (Lyon 1998). Therefore, the variations in the reported size are likely due to differences in polypeptide or lipid content. Since the light-harvesting antenna proteins are excluded from the crystals during their formation (Lyon et al. 1993) the precise polypeptide composition remains unknown. However, the presence of the minor antenna proteins (CP29, CP26, CP24 and PsbS) has been determined by direct immunogold-labeling techniques (Marr et al. 1996a). The stoichiometry of these proteins is difficult to determine but it seems probably that they are included in low-density areas between the dimers.

In addition, labeling with F_{ab} fragments (a fragment of papain-digested IgG antibody purified on protein A column) against the D1 and a α -cyt *b*₅₅₉ protein has been used to identify the position of the subunits in the crystal (Marr et al. 1996a). By considering the recent X-ray studies of cyanobacterial PS II (Zouni et al. 2001; Kamiya and Shen 2003) distinct positions both of the D1 and cyt *b*₅₅₉ proteins have been reported by Marr et al. (1996). From these results it should be concluded that immuno-gold labeling may not be the proper technique for localization of individual subunits within the crystals.

In spite of the fact that many biochemical and structural studies have provided conclusive evidences indicating a dimeric nature of PS II *in vivo* for both cyanobacteria and higher plants there are several contradictory statements suggesting PS II to be a monomer (see Nicholson et al. 1996). These results originate from an electron-crystallographic analysis of partially delipidated 2-D arrays obtained from spinach (Holzenburg et al. 1993; Rosenberg et al. 1997) and PS I-deficient barley mutant (*viridis* *zb*⁶³) grown in far-red light conditions (Stoylova et al. 1997, 2000).

Although projection maps of unit cells reported by Nicholson et al. (1996), Holzenburg et al. (1993) and Stoylova et al. (1997) are very similar to the dimeric PS II unit cells (see Table 1, and Figure 2[3a–c] and [3d–h]) the authors have suggested that each unit cell is constituted only by a monomeric PS II complex. A conclusion that PS II is present in native thylakoid membrane in its monomeric arrangement is distinctly inconsistent with all the data obtained by freeze-fracture/etching techniques, single particle analysis of detergent solubilized PS II preparations, and crystallographic analyses of 2-D and 3-D crystals. A comparison between the projection maps of dimeric PS II complex (Figure 2[2g, 3d–h, 4c, e]) with that reported as monomer (Figure 2[3a–c]) shows them to be very similar in their size and structural features. This suggests that the complex reported as PS II monomer is indeed represented by dimeric PS II complex. A little asymmetry observed, which is the main evidence for the PS II in monomeric state, is probably caused by an imperfect arrangement of protein subunits within the unit cell and could not be interpreted by a pseudo-twofold symmetrical arrangement of the D1/D2 and CP47/CP43 subunits as recently suggested by Ford et al. (2002). There is not a single feature in the density maps presented by Ford et al. (2002) that is recognizable and compatible with the data obtained by X-ray crystallography (Zouni et al. 2001; Kamiya and Shen 2003). The replacement of dimeric PS II by its monomeric counterpart is probably caused by a misinterpretation of the low-resolution EM data. Recent results clearly define that PS II is present in the thylakoid membrane as a dimer (Rögner et al. 1996; Barber 1998; Hankamer et al. 2001a).

2-D crystals from reconstituted particles

To obtain high-quality 2-D crystals PS II complexes can be purified and reconstituted with thylakoid lipids. Two types of PS II complex have been subjected

to crystallization trials: CP47-RC subcomplex (CP47-D1-D2-cyt *b*₅₅₉) and the PS II core complex.

2-D crystals of CP47-RC subcomplex have been prepared by several authors (Dekker et al. 1990; Nakazato et al. 1996; Mayanagi et al. 1998). A rectangular unit cell (approximately 17 × 15 nm) with *p*22₁2₁ plane group symmetry has been reported for the crystals. The unit cell contained four stain-excluding regions each designated to include one monomeric subcomplex. Two of them were oriented by their luminal sides and form a unit dimer with a two-fold rotational symmetry. The latter two formed a dimer with two-fold axis but the monomers were arranged by their stromal sides with an inverse handedness of the dimer with respect to the first dimer. Each monomer had an asymmetrical shape with dimensions of 10 × 7.5 nm and height of 6 nm with an estimated molecular mass of 150 kDa (Nakazato et al. 1996). A 3-D reconstruction of the monomeric PS II subcomplex revealed four luminal and three stromal domains (Mayanagi et al. 1998).

Specific arrangement of monomeric units within the crystal probably led to the misinterpretation of the results in the work of Rhee et al. (1997). A projection map of dimeric association of two CP47-RC monomers has been superimposed to central part of the projection map of the PS II-LHC II supercomplex obtained by Boekema et al. (1995). Although dimeric organization of PS II has been suggested it seems likely that dimeric association of CP47-RC subcomplex in 2-D crystals is unnatural and does not correspond to the native arrangement of the PS II subunits (see the paragraph above).

Recently, a 3-D model of helical arrangement of the CP47-RC subcomplex has been published (Rhee et al. 1998). 23 transmembrane α -helices have been identified, of which 16 have been assigned to major PS II subunits. Ten (5 × 2) transmembrane helices forming a roughly S-shaped feature in near twofold symmetry around a local axis were assigned to the D1/D2 heterodimer whereas an adjacent group of 3 × 2 helices to CP47 (Rhee 2001).

The 2-D crystals containing full PS II core complex have also been obtained (Tsiotis et al. 1996b; Morris et al. 1997; Hankamer et al. 2001b). As opposed to the previous reports they also contained the inner antenna protein CP43 and provided overall picture of subunit organization in the PS II core complex. The unit cell contained dimeric PS II complex with well-preserved twofold rotational symmetry around a central stain cavity and closely corresponded to the

dimeric PS II obtained by negative staining and single particle analysis (compare Figure 2[4b–d and 2g]). A superimposition of the projection map of CP47-RC subcomplex (Rhee et al. 1998) to each half of the PS II core dimer has revealed the location of CP43 subunit (Hankamer et al. 1999).

In contrast to spinach, 2-D crystals of dimeric PS II core complex isolated from *S. elongatus* have revealed different organization of a unit cell. The unit cell contained two PS II dimers in opposite orientation showing both luminal and stromal views of the complex to be a mirror image of each other (daFonseca et al. 2002). By comparing both models, the relative positioning of the main subunits, CP47, CP43, D2, D1, and cyt *b*₅₅₉, is essentially identical within each monomer, emphasizing the conservation of the basic structural features of PS II between higher plants and cyanobacteria.

Organization of the transmembrane helices

Electron and X-ray crystallography studies have revealed the organization of the transmembrane helices within PS II core complex of spinach (Rhee et al. 1998; Hankamer et al. 2001b), thermophilic cyanobacteria *Synechococcus elongatus* (Zouni et al. 2001) and *Thermosynechococcus vulcanus* (Kamiya and Shen 2003). Since the resolution is insufficient to resolve the atomic structure, the exact identification of the helices relies on comparisons with the known high-resolution structures of reaction centers of purple bacteria (Deisenhofer et al. 1985; Michel and Deisenhofer 1988) and PS I (Krauss et al. 1996; Fromme et al. 2001).

The overall organization of 22 transmembrane helices of the major PS II subunits (CP47, CP43, D1, D2) is very similar in higher plant and cyanobacterial PS II core complex. CP47 and CP43 are located on opposite sides of the centrally located D1/D2 proteins, related to each other by pseudo-twofold rotation axis. The structural similarity of the helix organization of the D1/D2 proteins with the L/M subunits of the purple bacterial RC and the D1/CP43 and D2/CP47 clusters with that of the PsaA and PsaB proteins of the reaction center of PS I supports the hypothesis about evolutionary relationships between these three types of reaction centers in photosynthetic organism (Schubert et al. 1998; Heathcote et al. 2002).

In addition, further 12 (spinach) and 14 (cyanobacteria) transmembrane helices have been identified in

each monomer of the PS II core dimer and assigned to the PS II low molecular weight subunits. Two of them (PsbE and PsbF, as components of *cyt b*₅₅₉) have been unambiguously identified by locating the haem group near to D2 protein of D1/D2 reaction center complex (Zouni et al. 2001; Kamiya and Shen 2003). The assignment of the others is still uncertain, either based on cross-linking (Harrer et al. 1998; Büchel et al. 2001; Lupinkova et al. 2002) or immunogold-labeling experiments (Büchel et al. 2001) and recently discussed by Hankamer et al. (2001a).

In order to identify the location of the PS II low molecular weight subunits two possible ways have been outlined: (i) electron microscopy of His-tagged PS II particles labeled with Ni²⁺-NTA gold cluster (Büchel et al. 2001), and (ii) comparative studies of wild-type and mutants depleted of the small PS II subunits (Shi et al. 2000; Komenda et al. 2001; Swiatek et al. 2001).

Organization of the extrinsic subunits

There are 3–4 extrinsic proteins associated with luminal side of PS II, which play important roles in maintaining the function and stability of the oxygen-evolving complex (OEC). Among the extrinsic proteins, only the 33 kDa protein is common to all of the organism. Higher plants and green algae, in addition, contain the 23 kDa and 16 kDa extrinsic subunits coded by *psbP* and *psbQ* gene, respectively. In cyanobacteria these proteins are missing and they are replaced by another two proteins coded by *psbU* (12-kDa protein) and *psbV* gene (*cyt c*₅₅₀). The extrinsic proteins protrude from thylakoid membrane and can be easily removed by various salt washing. A comparison of difference maps of salt-washed with non-washed preparations have enabled us to identify location of luminal subunits.

The location of the 33 kDa extrinsic protein has been determined in various PS II complexes from both higher plants and cyanobacteria (Haag et al. 1990; Boekema et al. 1995; Hasler et al. 1997; Boekema et al. 2000b). The side-view projection difference maps (+/– 33 kDa) of non-washed and Tris-washed of both higher plants and cyanobacterial dimeric PS II core complex showed two separated protrusions (Figure 3E), symmetrically located with respect to the center of the complex (Boekema et al. 1995; Kühl et al. 1999). Only a single central protrusion has been determined for the side-view of dimeric PS II-LHC II

supercomplex (Boekema et al. 1995, 1998a). As can be seen in Figures 3A and B, the single central protrusion observed in the PS II-LHC II supercomplex is composed by an overlap of two 33 kDa subunits. Moreover, the difference image of top-view projection maps after a removal of the 33 kDa protein have resulted in two regions of density change within the supercomplex indicating the presence of two 33 kDa subunits within the dimeric supercomplex (Boekema et al. 2000b). Although there are some biochemical evidences that two copies of 33 kDa protein exists per one PS II reaction center (Xu and Bricker 1992) these structural studies ruled out this possibility and have indicate a ratio of 1:1 between the 33 kDa and the PS II reaction center (Figure 3B).

In order to preserve the binding of additional extrinsic proteins the presence of 1 M glycine betaine has resulted in the PS II-LHC II supercomplex retaining the 23 kDa extrinsic protein (Boekema et al. 1998a). The side-view projection of the supercomplex showed two additional protein densities flanking the central protrusion (Figure 3A). Together with difference mapping of top-view projections (+/– 23 kDa) of the supercomplex the location of the 23 kDa subunit has been indicated (Figure 3B).

Recently, a 3-D reconstruction of the PS II-LHC II supercomplexes from spinach (Nield et al. 2000b, 2002) and *C. reinhardtii* (Nield et al. 2000a) have revealed all the three extrinsic subunits (33, 23 and 16 kDa) as four protrusions extending to 5–6 nm above the luminal surface. In accordance with the data obtained from single particle analysis (Boekema et al. 1998a) two centrally located luminal protrusions have been assigned contain the 33 kDa extrinsic subunit whereas two more extensive protrusions have been suggested to accommodate both the 23 kDa and 16 kDa subunit (Nield et al. 2000a). The stoichiometry ratio of the three extrinsic proteins (PsbO, PsbP, and PsbQ) is 1:1:1 and tetrameric organization of the extrinsic subunits at the luminal surface of the supercomplex closely resembles to that obtained earlier by freeze-etching studies (Seibert et al. 1987).

Location of additional cyanobacterial OEC subunits (*cyt c*₅₅₀ and 12 kDa) has been studied by single particle analysis of negatively stained preparations (Boekema et al. 1995; Kühl et al. 1999; Nield et al. 2000a). The side-view projection revealed an overall height of the particle to be 9.5 nm with other additional protrusions aside to the two central protrusions (Figure 3E). The central protrusions had been previously identified as the 33 kDa subunits, while the lower parts

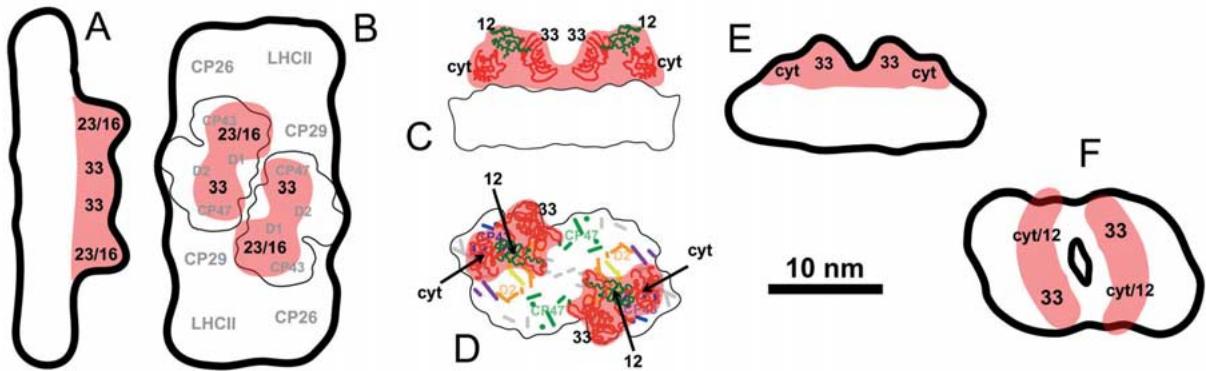


Figure 3. Schematic representation in subunit organization of the extrinsic subunits on the luminal side of dimeric PS II in higher plants (A, B) and cyanobacteria (C–F). The location of the extrinsic subunits is indicated by red.

A. and B. Side-view (A) and top-view (B) projection maps of the spinach PS II-LHC II supercomplex obtained by cryo-EM and 3-D reconstitution (Nield et al. 2000b). The contour of the spinach dimeric PS II core complex (Hankamer et al. 1999) is overlaid.

C. and D. Side-view (C) and top-view (D) projection maps of cyanobacterial dimeric PS II core complex obtained by X-ray crystallography. The coordinates are taken from Protein Data Bank, code 1FE1 (Zouni et al. 2001) and 1IZL (Kamiya and Shen 2003). The C α backbone of the 33 kDa and cyt *c*₅₅₀ (dark red) and 12 kDa subunits (dark green) are indicated. The underlying transmembrane α -helices are represented by columns and the assignment of individual proteins are depicted in different colours (D1, yellow; D2 orange; CP47, green; CP43, blue; cyt *b*₅₅₉, purple; unidentified helices, grey).

E. Side-view projection contours of negatively stained cyanobacterial PS II core complex obtained by EM and single particle analysis (Kühl et al. 1999).

F. Top-view projection contours of cyanobacterial PS II core complex obtained by cryo-EM and 3-D reconstitution (Nield et al. 2000a). Note the differences in the organization of the OEC subunits between the data obtained from X-ray and 3-D single particle image reconstruction (D and F). On the other hand, the data from single particle analysis of the side-view projection (E) indicates the position of the cyt *c*₅₅₀ to that obtained by X-ray crystallography. See text for details. The scale bar measures 10 nm.

of outer protrusions have been assigned to the cyt *c*₅₅₀ subunits (Kühl et al. 1999). From the observation that the 12 kDa subunit can only bind to PS II in the presence of both 33 kDa and cyt *c*₅₅₀ subunits (Shen and Inoue 1993; Shen et al. 1997), the upper part of the outer protrusions was suggested to be occupied by the 12 kDa subunit.

A 3-D reconstruction of cyanobacterial dimeric PS II complex revealed the OEC subunits as a tetrameric particle extending to 2.5 nm above the luminal surface (Nield et al. 2000a). Two protrusions have been assigned to the 33 kDa subunit whereas the latter ones to the cyt *c*₅₅₀/12-kDa subunits (Figure 3F). The similarities in the arrangements of cyanobacterial and higher plant OEC subunits led authors to the conclusion that structural pattern of the OEC proteins seems to be a basic structural feature of the PS II core complex and is conserved among the higher plants, green algae and cyanobacteria (Nield et al. 2000a).

Recently, structural data derived from the X-ray diffraction analysis of the PS II crystal have revealed the position of the 33 kDa, cyt *c*₅₅₀ (Zouni et al. 2001) and 12 kDa subunits (Kamiya and Shen 2003). Top-view projection map of the dimeric PS II core complex indicates the 33 kDa protein located towards the N-

terminus of the D1 protein and luminal loops of CP47 of the neighboring PS II monomer (Figure 3D). Cyt *c*₅₅₀ is situated close to the shorter edge of the complex over luminal loops of CP43 and heterodimeric cyt *b*₅₅₉ (Zouni et al. 2001). The 12 kDa protein is located between the 33 kDa protein and cyt *c*₅₅₀ but apart from the luminal surface of the membrane by approximately 3 nm (Kamiya and Shen 2003).

By comparing X-ray data (Kamiya and Shen 2003) with 3-D model of negatively stained PS II core complex (Nield et al. 2000a) distinct locations of both 33 kDa and cyt *c*₅₅₀ subunits can be observed (compare Figures 3D and 3F). Although it has been previously suggested that the location of cyanobacterial 33 kDa subunit corresponds to that of higher plants (Nield et al. 2000a), X-ray data located the 33 kDa subunit to an area where the mass of the higher plant 23/16 kDa subunits has been attributed. On the top of that X-ray data shifted the location of the cyt *c*₅₅₀ to an outer edge of the complex (Zouni et al. 2001). However, the arrangement of cyanobacterial OEC subunits in the crystals well corresponds to the results of Kühl et al. (1999). The side-view projection map of cyanobacterial PS II core complex revealed two cyt *c*₅₅₀ protrusions with the distance of 14 nm which is in a good agreement

with the location of the cyt *c*₅₅₀ subunits in the crystal at the outer edge of the complex (Figure 3E).

As the 33 kDa extrinsic subunit is a manganese-stabilizing protein commonly found and highly conserved (45%) in all oxygenic photosynthetic organisms (see Seidler 1996; Bricker and Frankel 1998), it would be expected that the subunits have similar structure and location in both systems. This assumption has been supported by cross-reconstruction experiments showing that the 33 kDa protein from *Arabidopsis* can bind to PS II from spinach (Betts et al. 1994), and that it can be exchanged between the cyanobacteria and higher plants (Koike and Inoue 1985) as well as among cyanobacteria, red algae and higher plants (Enami et al. 2000). On the other hand, it should be noted that several discrepancies in binding properties of the extrinsic components exists. A PsbO⁻ mutant of *Synechocystis* 6803 can grow photoautotrophically (Mayes et al. 1991; Philbrick et al. 1991) whereas in identical mutant of *Ch. reinhardtii* was inhibited in photoautotrophic growth (Mayfield et al. 1987). In contrast to higher plant where NaCl-wash effectively releases the 16 and 23 kDa proteins (Kuwabara and Murata 1982) cyanobacterial subunits are not dissociated from PS II by NaCl treatment (Shen et al. 1992; Enami et al. 1995). The 23 kDa protein of higher plants can bind to the PS II core complex only in the presence of 33 kDa protein (Andersson et al. 1984) whereas the cyanobacterial cyt *c*₅₅₀ can bind to PS II by itself (Shen and Inoue 1993; Shen et al. 1995). The absence of 23 and 16 kDa subunits in higher plants can be substituted by Ca²⁺ and Cl⁻ ions (see Debus 1992). This is different from what was seen in cyanobacterial PS II where the absence or presence of cyt *c*₅₅₀ and the 12-kDa protein does not have a direct correlation with the requirement of Ca²⁺ and Cl⁻ for oxygen evolution (Shen and Inoue 1993; Shen et al. 1997).

Distinct location of the 33 kDa subunits is also in agreement with a cross-linking data indicating close vicinity to the CP47 and the D1/D2 heterodimer (see Seidler 1996; Bricker and Frankel 1998). Although it has been accepted that the 33 kDa subunit is bound to only one reaction center of PS II, the X-ray structural model (Zouni et al. 2001) does not exclude the possibility that part of the 33 kDa protein can bind the CP47 in the other monomer unit within the dimeric PS II (Figure 3D).

The possible explanation for the distinct arrangement of the OEC subunits between the cyanobacteria and higher plants should be outlined. The eukaryotic red algal PS II complex contains, in addition to three

cyanobacterial extrinsic proteins, a 20 kDa protein which is found neither in cyanobacterial nor in higher plants (Enami et al. 1995). Enami et al. (1998) proposed hypothesis that during evolution of the OEC from prokaryotic cyanobacteria to eukaryotic red algae, cyt *c*₅₅₀ was changed so that it can no longer bind to PS II itself. Concomitant with this, the 20 kDa protein was newly developed in order to keep binding of the cyt *c*₅₅₀ to PS II complex. During evolution from the red algae to higher plants, however, cyt *c*₅₅₀ was replaced by the 23 kDa protein which does not require the 20 kDa protein for binding (Enami et al. 1998). These changes could have resulted in a different organization of the OEC polypeptides at the luminal side of PS II.

Conclusion

Electron microscopy is a powerful technique to study the structure of proteins and membranes at a low- and high-resolution. Low-resolution data are obtained by application of freeze-fracture/etching techniques and/or negative staining with single particle analysis. These methods give us useful information on the overall size and distribution of the PS II and LHC II complexes within the thylakoid membrane. 2-D cryo-electron crystallography approach has been also shown to provide high-resolution structural data. EM analysis of 2-D crystals has revealed a 3-D model of LHC II complex (Kühlbrandt et al. 1994) and recently the PS II core complex (Hankamer et al. 2001b). Electron crystallography provides structural information of PS II that are very consistent with the data derived by X-ray crystallography of 3-D crystals. In conclusion, combination of EM techniques with X-ray crystallography could contribute to the understanding of not only the structural properties of PS II but also to its interactions with other photosynthetic complexes within the thylakoid membrane of higher plants and cyanobacteria.

Acknowledgements

We are very grateful to Drs Josef Komenda and Pavel Šiffel for helpful discussions and their critical reading of the manuscript. We also acknowledge financial support from the grants No LN00A141 and FRVS 1292/2002 of the Ministry of Education, Youth and Sports of the Czech Republic.

References

Andersson B and Akerlund HE (1978) Inside-out membrane vesicles isolated from spinach thylakoid. *Biochim Biophys Acta* 503: 462–472

Andersson B and Anderson JM (1980) Lateral heterogeneity in the distribution of chlorophyll–protein complexes of the thylakoid membranes of spinach chloroplasts. *Biochim Biophys Acta* 593: 427–440

Andersson B, Larsson C, Jansson C, Ljungberg U and Akerlund HE (1984) Immunological studies on the organization of proteins in photosynthetic oxygen evolution. *Biochim Biophys Acta* 766: 21–28

Armond PA, Staehelin LA and Arntzen CJ (1977) Spatial relationship of Photosystem I, Photosystem II, and the light-harvesting complex in chloroplast membranes. *J Cell Biol* 73: 400–418

Aro EM, Virgin I and Andersson B (1993) Photoinhibition of Photosystem II. Inactivation, protein damage and turnover. *Biochim Biophys Acta* 1143: 113–134

Arvidsson PO and Sundby C (1999) A model for the topology of the chloroplast thylakoid membrane. *Aust J Plant Physiol* 26: 687–694

Baena-Gonzales E and Aro EM (2002) Biogenesis, assembly and turnover of Photosystem II units. *Philos Trans R Soc London B Biol Sci* 357: 1451–1459

Bald D, Kruip J and Rogner M (1996) Supramolecular architecture of cyanobacterial thylakoid membranes: how is the phycobilisomes connected with the photosystems? *Photosynth Res* 49: 103–118

Barber J (1998) Photosystem two. *Biochim Biophys Acta* 1365: 269–277

Barber J and Andersson B (1992) Too much of a good thing – light can be bad for photosynthesis. *Trends Biochem Sci* 17: 61–66

Barber J, Morris E and Büchel C (2000) Revealing the structure of the Photosystem II chlorophyll binding proteins, CP43 and CP47. *Biochim Biophys Acta* 1459: 239–247

Barber J, Nield J, Morris EP, Zheleva D and Hankamer B (1997) The structure, function and dynamics of Photosystem II. *Physiol Plant* 100: 817–827

Bassi R, Magaldi AG, Tognon G, Giacometti GM and Miller KR (1989) Two-dimensional crystals of the Photosystem II reaction center complex from higher plants. *Eur J Cell Biol* 50: 84–93

Bassi R, Marquardt J and Lavergne J (1995) Biochemical and functional properties of Photosystem II in agranal membranes from maize mesophyll and bundle-sheath chloroplasts. *Eur J Biochem* 233: 709–719

Berthold DA, Babcock GT and Yocum CF (1981) A highly resolved, oxygen-evolving PS II preparation from spinach thylakoid membranes – electron-paramagnetic resonance and electron transport properties. *FEBS Lett* 134: 231–234

Betts SD, Hachigian TM, Pichersky E and Yocum CF (1994) Reconstitution of the spinach oxygen-evolving complex with recombinant *Arabidopsis* manganese-stabilizing protein. *Plant Mol Biol* 26: 117–130

Boekema EJ, Boonstra AF, Dekker JP and Rogner M (1994) Electron microscopical structural analysis of Photosystem I, Photosystem II and the cytochrome-*b*₆/f complex from green plants and cyanobacteria. *J Bioenerg Biomembr* 26: 17–29

Boekema EJ, Hankamer B, Bald D, Kruip J, Nield J, Boonstra AF, Barber J and Rogner M (1995) Supramolecular structure of the Photosystem II complex from green plants and cyanobacteria. *Proc Natl Acad Sci USA* 92: 175–179

Boekema EJ, Nield J, Hankamer B and Barber J (1998a) Localization of the 23-kDa subunit of the oxygen evolving complex of Photosystem II by electron-microscopy. *Eur J Biochem* 252: 268–276

Boekema EJ, van Roon H and Dekker JP (1998b) Specific association of Photosystem II and light-harvesting complex II in partially solubilized Photosystem II membranes. *FEBS Lett* 424: 95–99

Boekema EJ, van Roon H, Calkoen F, Bassi R and Dekker JP (1999a) Multiple types of association of Photosystem II and its light-harvesting antenna in partially solubilized Photosystem II membranes. *Biochemistry* 38: 2233–2239

Boekema EJ, van Roon H, van Breemen JFL and Dekker JP (1999b) Supramolecular organization of Photosystem II and its light-harvesting antenna in partially solubilized Photosystem II membranes. *Eur J Biochem* 266: 444–452

Boekema EJ, van Breemen JFL, van Roon H and Dekker JP (2000a) Arrangement of Photosystem II supercomplexes in crystalline macrodomains within the thylakoid membrane of green plant chloroplasts. *J Mol Biol* 301: 1123–1133

Boekema EJ, van Breemen JFL, van Roon H and Dekker JP (2000b) Conformational changes in Photosystem II supercomplexes upon removal of extrinsic subunits. *Biochemistry* 39: 12907–12915

Branton DS, Bullivant NB, Gilula MJ, Karnovsky H, Moor K, Mühlthaler DH, Northcote L, Packer B, Satir P, Satir V, Speth LA, Staehelin LA, Steere RL and Weinstein RS (1975) Freeze-etching nomenclature. *Science* 190: 54–56

Bricker TM (1990) The structure and function of CPA-1 and CPA-2 in Photosystem II. *Photosynth Res* 24: 1–13

Bricker TM and Frankel LK (1998) The structure and function of the 33 kDa extrinsic protein of Photosystem II: a critical assessment. *Photosynth Res* 56: 157–173

Bricker TM and Frankel LK (2002) The structure and function of CP47 and CP43 in Photosystem II. *Photosynth Res* 72: 131–146

Büchel C, Morris E, Orlova E and Barber J (2001) Localization of the PsbH subunit in Photosystem II: a new approach using labeling of His-tags with a Ni²⁺-NTA gold cluster and single particle analysis. *J Mol Biol* 312: 371–379

Dainese P and Bassi R (1991) Subunit stoichiometry of the chloroplast Photosystem II antenna system and aggregation state of the component chlorophyll *a/b* binding proteins. *J Biol Chem* 266: 8136–8142

Debus RJ (1992) The manganese and calcium ions of photosynthetic oxygen evolution. *Biochim Biophys Acta* 1102: 269–352

Deisenhofer J, Epp O, Miki K, Huber R and Michel H (1985) Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution. *Nature* 318: 618–624

Dekker JP, Boekema EJ, Witt HT and Rogner M (1988) Refined purification and further characterization of oxygen-evolving and Tris-treated Photosystem II particles from the thermophilic cyanobacterium *Synechococcus* sp. *Biochim Biophys Acta* 936: 307–318

Dekker JP, Betts SD, Yocum CF and Boekema EJ (1990) Characterization by electron microscopy of isolated particles and two-dimensional crystals of the CP47-D1-D2-cytochrome *b*₅₅₉ complex of Photosystem II. *Biochemistry* 29: 3220–3225

Dekker JP, Germano M, van Roon H and Boekema EJ (2002) Photosystem II solubilizes as a monomer by mild detergent treatment of unstacked thylakoid membranes. *Photosynth Res* 72: 203–210

Dunahay TG, Staehelin LA, Seibert M, Ogilvie PD and Berg S (1984) Structural, biochemical and biophysical characterization of four oxygen-evolving Photosystem II preparation from spinach. *Biochim Biophys Acta* 764: 179–193

Eijckelhoff C, Dekker JP and Boekema EJ (1997) Characterization by electron microscopy of dimeric Photosystem II core com-

plexes from spinach with and without CP43. *Biochim Biophys Acta* 1321: 10–20

Enami I, Murayama H, Ohta H, Kamo M, Nakazato K and Shen JR (1995) Isolation and characterization of a Photosystem II complex from the red alga *Cyanidium caldarium*: association of cytochrome *c*₅₅₀ and a 12 kDa protein with the complex. *Biochim Biophys Acta* 1232: 208–216

Enami I, Kikuchi S, Fukuda T, Ohta H and Shen JR (1998) Binding and functional properties of four extrinsic proteins of Photosystem II from a red alga, *Cyanidium caldarium*, as studied by release – reconstitution experiments. *Biochemistry* 37: 2787–2793

Enami I, Yoshihara S, Tohri A, Okumura A, Ohta H and Shen JR (2000) Cross-reconstitution of various extrinsic proteins and Photosystem II complexes from cyanobacteria, red algae and higher plants. *Plant Cell Physiol* 41: 1354–1364

Ford RC, Rosenberg MF, Shepherd FH, McPhie P and Holzenburg A (1995) Photosystem II 3-D structure and the role of the extrinsic subunits in photosynthetic oxygen evolution. *Micron* 26: 133–140

Ford RC, Stoylova SS and Holzenburg A (2002) An alternative model for Photosystem II/light-harvesting complex II in grana membranes based on cryo-electron microscopy studies. *Eur J Biochem* 269: 326–336

Fromme P, Jordan P and Krauss N (2001) Structure of Photosystem I. *Biochim Biophys Acta* 1507: 5–31

Fujita Y, Murakami A, Aizawa K and Ohki K (1995) Short-term and long-term adaptation of the photosynthetic apparatus: homeostatic properties of thylakoids. In: Bryant DA (ed) *The Molecular Biology of Cyanobacteria*, pp 677–692. Kluwer Academic Publishers, Dordrecht, The Netherlands

Gantt E (1994) Supramolecular membrane organization. In: Bryant DA (ed) *The Molecular Biology of Cyanobacteria*, pp 139–216. Kluwer Academic Publishers, Dordrecht, The Netherlands

Ghanotakis DF and Yocom CF (1985) Polypeptides of Photosystem II and their role in oxygen evolution. *Photosynth Res* 7: 97–114

Ghanotakis DF and Yocom CF (1990) Photosystem II and the oxygen-evolving complex. *Ann Rev Plant Physiol Plant Mol Biol* 41: 255–276

Giddings TH and Staehelin LA (1979) Changes in thylakoid structure associated with the differentiation of heterocysts in the cyanobacterium *Anabaena cylindrica*. *Biochim Biophys Acta* 546: 373–382

Giddings TH, Wasmann C and Staehelin LA (1983) Structure of the thylakoids and envelope membranes of the cyanelles of *Cyanophora paradoxa*. *Plant Physiol* 71: 409–419

Glazer AN (1982) Phycobilisomes: structure and dynamics. *Ann Rev Microbiol* 36: 173–198

Gray MW (1992) The endosymbiont hypothesis revisited. *Int Rev Cytol* 141: 233–357

Green BR and Durnford DG (1996) The chlorophyll-carotenoid proteins of oxygenic photosynthesis. *Ann Rev Plant Physiol Plant Mol Biol* 47: 685–714

Green BR, Pichersky E and Kloppstech K (1991) Chlorophyll *a/b*-binding proteins: an extended family. *Trends Biochem Sci* 16: 181–186

Haag E, Irrgang KD, Boekema EJ and Renger G (1990) Functional and structural analysis of Photosystem II core complexes from spinach with high oxygen evolution capacity. *Eur J Biochem* 189: 47–53

Hainfeld JF, Liu WQ, Halsey CMR, Freimuth P and Powell RD (1999) Ni-NTA-gold clusters target his-tagged proteins. *J Struct Biol* 127: 185–198

Hankamer B, Boekema EJ and Barber J (1997a) Structure and membrane organization of Photosystem II in green plants. *Ann Rev Plant Physiol Plant Mol Biol* 48: 641–671

Hankamer B, Nield J, Zheleva D, Boekema E, Jansson S and Barber J (1997b) Isolation and biochemical characterization of monomeric and dimeric Photosystem II complexes from spinach and their relevance to the organization of Photosystem II *in vivo*. *Eur J Biochem* 243: 422–429

Hankamer B, Morris EP and Barber J (1999) Revealing the structure of the oxygen-evolving core dimer of Photosystem II by cryo-electron crystallography. *Nat Struct Biol* 6: 560–564

Hankamer B, Morris EP, Nield J, Carne A and Barber J (2001a) Subunit positioning and transmembrane helix organisation in the core dimer of Photosystem II. *FEBS Lett* 504: 142–151

Hankamer B, Morris EP, Nield J, Gerle C and Barber J (2001b) Three-dimensional structure of the Photosystem II core dimer of higher plants determined by electron microscopy. *J Struct Biol* 135: 262–269

Hansson O and Wydrzynski T (1990) Current perceptions of Photosystem II. *Photosynth Res* 23: 131–162

Harrer R, Bassi R, Testi MG and Schafer C (1998) Nearest-neighbor analysis of a Photosystem II complex from *Marchantia polymorpha* L. (liverwort), which contains reaction center and antenna proteins. *Eur J Biochem* 255: 196–205

Hasler L, Ghanotakis D, Fedtke B, Spyridaki A, Miller M, Muller SA, Engel A and Tsiotis G (1997) Structural analysis of Photosystem II: comparative study of cyanobacterial and higher plant Photosystem II complexes. *J Struct Biol* 119: 273–283

Heathcote P, Fyfe PK and Jones MR (2002) Reaction centres: the structure and evolution of biological solar power. *Trends Biochem Sci* 27: 79–87

Holzenburg A, Bewly MC, Wilson FH, Nicholson WV and Ford RC (1993) Three-dimensional structure of Photosystem II. *Nature* 363: 470–472

Ikeuchi M (1992) Subunit proteins of Photosystem II. *Bot Mag Tokyo* 105: 327–373

Irrgang KD, Boekema EJ, Vater J and Renger G (1988) Structural determination of the Photosystem II core complex from spinach. *Eur J Biochem* 178: 209–217

Jansson S (1994) The light-harvesting *a/b*-binding proteins. *Biochim Biophys Acta* 1184: 1–19

Jansson S, Stefansson H, Nyström U, Gustafsson P and Albertsson PA (1997) Antenna protein composition of PS I and PS II in thylakoid sub-domains. *Biochim Biophys Acta* 1320: 297–309

Kamiya N and Shen JR (2003) Crystal structure of oxygen-evolving Photosystem II from *Thermosynechococcus vulcanus* at 3.7 Å resolution. *Proc Natl Acad Sci USA* 100: 98–103

Kitmitto A, Mustafa AO, Ford JW, Holzenburg A and Ford RC (1999) Does photoinhibition and/or phosphorylation of Photosystem II influence its *in vivo* oligomeric state? *Biochim Biophys Acta* 1413: 21–30

Koike H and Inoue Y (1985) Properties of a peripheral 34 kDa protein in *Synechococcus vulcanus* Photosystem II particles – its exchanability with spinach 33 kDa protein in reconstitution of O₂ evolution. *Biochim Biophys Acta* 807: 64–73

Komenda J, Lupinkova L and Kopecky J (2002) Absence of the psbH gene product destabilizes Photosystem II complex and bicarbonate binding on its acceptor side in *Synechocystis* PCC 6803. *Eur J Biochem* 269: 610–619

Krauss N, Schubert WD, Klukas O, Fromme P, Witt HT and Saenger W (1996) Photosystem I at 4 Å resolution represents the first structural model of a joint photosynthetic reaction centre and core antenna system. *Nat Struct Biol* 3: 965–973

Kruse O (2001) Light-induced short-term adaptation mechanisms under redox control in the PS II-LHC II supercomplex: LHC II state transitions and PS II repair cycle. *Naturwissenschaften* 88: 284–292

Kühl H, Rogner M, van Breemen JFL and Boekema EJ (1999) Localization of cyanobacterial Photosystem II donor-side subunits by electron microscopy and the supramolecular organization of Photosystem II in the thylakoid membrane. *Eur J Biochem* 266: 453–459

Kühlbrandt W, Wang DN and Fujiyoshi Y (1994) Atomic model of plant light-harvesting complex by electron crystallography. *Nature* 367: 614–21

Kuwabara T and Murata N (1982) Inactivation of photosynthetic oxygen evolution and concomitant release of three polypeptides in the Photosystem II particles of spinach chloroplasts. *Plant Cell Physiol* 23: 533–539

Lacambra M, Larsen U, Olive J, Bennoun P and Wollman FA (1984) Characterization of the thylakoid membranes of wild-type and mutants of *Chlorella sorokiniana*. *Photobiochem Photobiophys* 8: 191–205

Lupinkova L, Metz JG, Diner BA, Vass I and Komenda J (2002) Histidine residue 252 of the Photosystem II D1 polypeptide is involved in a light-induced cross-linking of the polypeptide with the alpha subunit of cytochrome b_{559} : study of a site-directed mutant of *Synechocystis* PCC 6803. *Biochim Biophys Acta* 1554: 192–201

Lyon MK (1998) Multiple crystal types reveal Photosystem II to be a dimer. *Biochim Biophys Acta* 1364: 403–419 Lyon MK, Marr KM and Furciniti PS (1993) Formation and characterization of two-dimensional crystals of Photosystem II. *J Struct Biol* 110: 133–140

MacColl R (1998) Cyanobacterial phycobilisomes. *J Struct Biol* 124: 311–334

Mamedov F, Stefansson H, Albertsson PA and Styring S (2000) Photosystem II in different parts of the thylakoid membrane: a functional comparison between different domains. *Biochemistry* 39: 10478–10486

Marquardt J, Mörschel E, Rhie E and Westermann M (2000) Ultrastructure of *Acaryochloris marina*, an oxyphotobacterium containing mainly chlorophyll d. *Arch Microbiol* 174: 181–188

Marr KM, Mastronarde DN and Lyon MK (1996a) Two-dimensional crystals of Photosystem II: biochemical characterization, cryoelectron microscopy and localization of the D1 and cytochrome b_{559} polypeptides. *J Cell Biol* 132: 823–833

Marr KM, McFeeters RL and Lyon MK (1996b) Isolation and structural analysis of two-dimensional crystals of Photosystem II from *Hordeum vulgare viridis* zb^{63} . *J Struct Biol* 117: 86–98

Mayanagi K, Ishikawa T, Toyoshima C, Inoue Y and Nakazato K (1998) Three-dimensional electron microscopy of the Photosystem II core complex. *J Struct Biol* 123: 211–224

Mayes SR, Cook KM, Self SJ, Zhang ZH and Barber J (1991) Deletion of the gene encoding the Photosystem II 33-kDa protein from *Synechocystis* sp. PCC 6803 does not inactivate water splitting but increases vulnerability to photoinhibition. *Biochim Biophys Acta* 1060: 1–12

Mayfield SP, Bennoun P and Rochaix JD (1987) Expression of the nuclear encoded OEE1 protein is required for oxygen evolution and stability of Photosystem II particles in *Chlamydomonas reinhardtii*. *EMBO J* 6: 313–318

McFadden GI (1999) Endosymbiosis and evolution of the plant cell. *Curr Opin Plant Biol* 2: 513–519

Melis A (1991) Dynamics of photosynthetic membrane composition and function. *Biochim Biophys Acta* 1058: 87–106

Michel H and Deisenhofer J (1988) Relevance of the photosynthetic reaction center from purple bacteria to the structure of Photosystem II. *Biochemistry* 27: 1–7

Miller KR and Cushman RA (1979) A chloroplast membrane lacking Photosystem II: Thylakoid stacking in the absence of the Photosystem II particle. *Biochim Biophys Acta* 546: 481–497

Miller KR, Miller GJ and McIntyre KR (1976) The light-harvesting chlorophyll–protein complex of Photosystem II: Its location in the photosynthetic membrane. *J Cell Biol* 71: 624–638

Morris EP, Hankamer B, Zheleva D, Friso G and Barber J (1997) The three-dimensional structure of a Photosystem II core complex determined by electron crystallography. *Structure* 5: 837–849

Mörschel E (1991) The light-harvesting antennae of cyanobacteria and red algae. *Photosynthetica* 25: 137–144

Mörschel E and Mühlthaler K (1983) On the linkage of exoplasmic freeze-fracture particles to phycobilisomes. *Planta* 158: 451–457

Mörschel E and Schatz GH (1987) Correlation of Photosystem II complexes with exoplasmic freeze-fracture particles of thylakoids of the cyanobacterium *Synechococcus* sp. *Planta* 172: 145–154

Mullineaux CW (1999) The thylakoid membranes of cyanobacteria: structure, dynamics and function. *Aust J Plant Physiol* 26: 671–677

Murata N and Miyao M (1985) Extrinsic membrane proteins in the photosynthetic oxygen-evolving complex. *Trends Biochem Sci* 10: 122–124

Mustard L (1996) Development of thylakoid membrane stacking. In: Ort DR and Yocom CF (eds) *Oxygenic Photosynthesis – The Light Reactions*, pp 59–68. Kluwer Academic Publishers, Dordrecht, The Netherlands

Mustard L, Cunningham FX and Gantt E (1992) Photosynthetic membrane topography: quantitative *in situ* localization of Photosystems I and II. *Proc Natl Acad Sci USA* 89: 10021–10025

Nakazato K, Toyoshima C, Enami I and Inoue Y (1996) Two-dimensional crystallization and cryo-electron microscopy of Photosystem II. *J Mol Biol* 257: 225–232

Nanba O and Satoh K (1987) Isolation of a Photosystem II reaction center consisting of D1 and D2 polypeptides and cytochrome b_{559} . *Proc Natl Acad Sci USA* 84: 109–112

Neushul M (1971) Uniformity of the thylakoid structure in a red, brown and two blue-green algae. *J Ultrastruct Res* 37: 532–543

Nicholson WV, Ford RC and Holzenburg A (1996) A current assessment of Photosystem II structure. *Bioscience Rep* 16: 159–187

Nield J, Balsera M, De Las Rivas J and Barber J (2002) Three-dimensional electron cryo-microscopy study of the extrinsic domains of the oxygen-evolving complex of spinach. *J Biol Chem* 277: 15006–15012

Nield J, Kruse O, Ruprecht J, da Fonseca P, Büchel C and Barber J (2000a) Three-dimensional structure of *Chlamydomonas reinhardtii* and *Synechococcus elongatus* Photosystem II complexes allows for comparison of their oxygen-evolving complex organization. *J Biol Chem* 275: 27940–27946

Nield J, Orlova EV, Morris EP, Gowen B, van Heel M and Barber J (2000b) 3D map of the plant Photosystem II supercomplex obtained by cryoelectron microscopy and single particle analysis. *Nat Struct Biol* 7: 44–47

Nilsson F, Simpson DJ, Jansson C and Andersson B (1992) Ultrastructural and biochemical characterization of a *Synechocystis* 6803 mutant with inactivated *psbA* genes. *Arch Biochem Biophys* 295: 340–347

Olive J and Valon O (1991) Structural organisation of the thylakoid membrane: freeze-fracture and immunocytochemical analysis. *J Electr Micr Tech* 18: 360–374

Olive J and Wollman FA (1998) Supramolecular organization of the chloroplast and of the thylakoid membranes. In: Rochaix JD, Goldschmidt-Clermont M and Merchan S (eds) *The Molecular Biology of Chloroplast and Mitochondria in Chlamydomonas*, pp 233–254. Kluwer Academic Publishers, Dordrecht, The Netherlands

Olive J, Wollman FA, Bennoun P and Recouvreur M (1981) Ultrastructure of thylakoid membranes in *C. reinhardtii*: evidence for variations in the partition coefficient of the light-harvesting complex containing particles upon membrane fracture. *Arch Biochem Biophys* 208: 456–467

Olive J, Recouvreur M, Girardbascou J and Wollman FA (1992) Further identification of the exoplasmic face particles on the freeze-fractured thylakoid membranes – a study using double and triple mutants from *Chlamydomonas reinhardtii* lacking various Photosystem II subunits and the cytochrome b_6/f complex. *Eur J Cell Biol* 59: 176–186

Olive J, Ajlani G, Astier C, Recouvreur M and Vernotte C (1997) Ultrastructure and light adaptation of phycobilisome mutants of *Synechocystis* PCC 6803. *Biochim Biophys Acta* 1319: 275–282

Paulsen H (1995) Chlorophyll a/b -binding proteins. *Photochem Photobiol* 62: 367–382

Peter GF and Thornber JP (1991) Biochemical evidence that the higher plant Photosystem II core complex is organized as a dimer. *Plant Cell Physiol* 32: 1237–1250

Philbrick JB, Diner BA and Zilinskas BA (1991) Construction and characterization of cyanobacterial mutants lacking the manganese-stabilizing polypeptide of Photosystem II. *J Biol Chem* 266: 13370–13376

Rhee KH (2001) Photosystem II: the solid structural era. *Annu Rev Biophys Biomol* 30: 307–328

Rhee KH, Morris EP, Zheleva D, Hankamer B, Kuhlbrandt W and Barber J (1997) Two-dimensional structure of plant Photosystem II at 8 Å resolution. *Nature* 389: 522–526

Rhee KH, Morris EP, Barber J and Kuhlbrandt W (1998) Three-dimensional structure of the plant Photosystem II reaction centre at 8 Å resolution. *Nature* 396: 283–286

Rögner M, Boekema EJ and Barber J (1996) How does Photosystem 2 split water? The structural basis of efficient energy conversion. *Trends Biochem Sci* 21: 44–49

Rögner M, Dekker JP, Boekema EJ and Witt HT (1987) Size, shape and mass of the oxygen-evolving Photosystem II complex from the thermophilic cyanobacterium *Synechococcus* sp. *FEBS Lett* 219: 207–211

Rosenberg MF, Holzenburg A, Shepherd FH, Nicholson WV, Flint TD and Ford RC (1997) Rebinding of the extrinsic proteins of Photosystem II studied by electron microscopy and single particle alignment: an assessment with small two-dimensional ordered arrays of Photosystem II. *Biochim Biophys Acta* 1319: 119–132

Ruprecht J and Nield J (2001) Determining the structure of biological macromolecules by transmission electron microscopy, single particle analysis and 3-D reconstruction. *Prog Biophys Mol Biol* 75: 121–164

Sandonà D, Croce R, Pagano A, Crimi M and Bassi R (1998) Higher plants light-harvesting proteins. Structure and function as revealed by mutation analysis of either protein or chromophore moieties. *Biochim Biophys Acta* 1365: 207–214

Santini C, Tidu V, Tognon G, Magaldi AG and Bassi R (1994) Three-dimensional structure of the higher plant Photosystem II reaction center and evidence for its dimeric organization *in vivo*. *Eur J Biochem* 221: 307–315

Schubert WD, Klukas O, Saenger W, Witt HT, Fromme P and Krauss N (1998) A common ancestor for oxygenic and anoxic photosynthetic systems: a comparison based on the structural model of Photosystem I. *J Mol Biol* 280: 297–314

Seibert M, deWitt M and Staehelin A (1987) Structural localization of the O₂-evolving apparatus to multimeric (tetrameric) particles on the luminal surfaces of freeze-etched photosynthetic membranes. *J Cell Biol* 105: 2257–2265

Seidler A (1996) The extrinsic polypeptides of Photosystem II. *Biochim Biophys Acta* 1277: 35–60

Shen JR and Inoue Y (1993) Binding and functional properties of two new extrinsic components, cytochrome c_{550} and 12-kDa protein, in cyanobacterial Photosystem II. *Biochemistry* 32: 1825–1832

Shen JR, Ikeuchi M and Inoue Y (1992) Stoichiometric association of extrinsic cytochrome c_{550} and 12-kDa protein with a highly purified oxygen-evolving Photosystem II core complexes from *Synechococcus vulcanus*. *FEBS Lett* 301: 145–149

Shen JR, Burnap RL and Inoue Y (1995) An independent role of cytochrome c_{550} in cyanobacterial Photosystem II as revealed by double deletion mutagenesis of the *psbO* and *psbV* genes in *Synechocystis* sp. PCC 6803. *Biochemistry* 34: 12661–12668

Shen JR, Ikeuchi M and Inoue Y (1997) Analysis of the *psbU* gene encoding the 12 kDa extrinsic protein of Photosystem II and studies on its role by deletion mutagenesis in *Synechocystis* sp. PCC 6803. *J Biol Chem* 272: 17821–17826

Sherman DM, Troyan TA and Sherman LA (1994) Localization of membrane proteins in the cyanobacterium *Synechococcus* sp. PCC 7942 – radial asymmetry in the photosynthetic complexes. *Plant Physiol* 106: 251–262

Shi LX, Lorkovic ZI, Oelmüller R and Schroder WP (2000) The low molecular mass PsbW protein is involved in the stabilization of the dimeric Photosystem II complex in *Arabidopsis thaliana*. *J Biol Chem* 275: 37945–37950

Simpson DJ (1978) Freeze-fracture studies on barley plastid membranes. II. Wild-type chloroplast. *Carlsberg Res Commun* 43: 365–389

Simpson DJ (1979) Freeze-fracture studies on barley plastid membranes. III. Location of the light-harvesting chlorophyll protein. *Carlsberg Res Commun* 44: 305–336

Simpson DJ and Andersson B (1986) Extrinsic polypeptides of the chloroplast oxygen evolving complex constitute the tetrameric ESs particles of higher plant thylakoids. *Carlsberg Res Commun* 51: 467–474

Sprague SG, Camm EL, Green BR and Staehelin LA (1985) Reconstitution of light-harvesting complexes and Photosystem II cores into galactolipid and phospholipid liposomes. *J Cell Biol* 100: 552–557

Staehelin LA (1975) Chloroplast membrane structure: intramembranous particles of different sizes make contact in stacked membrane regions. *Biochim Biophys Acta* 408: 1–11

Staehelin LA (1976) Reversible particle movements associated with unstacking and restacking of chloroplast membranes *in vitro*. *J Cell Biol* 71: 136–158

Staehelin LA and Arntzen CJ (1983) Regulation of chloroplast membrane function: protein phosphorylation changes the spatial organization of membrane components. *J Cell Biol* 97: 1327–1337

Staehelin LA and deWitt M (1984) Correlation of structure and function of chloroplast membranes at the supramolecular level. *J Cell Biochem* 24: (3) 261–269

Staehelin LA and van der Staay GWM (1996) Structure, composition, functional organisation and dynamics properties of thylakoid membranes. In: Ort DR and Yocum CF (eds) Oxygenic Photosynthesis – the Light Reactions, pp 11–30. Kluwer Academic Publishers, Dordrecht, The Netherlands

Stoylova SS, Flint TD, Ford RC and Holzenburg A (1997) Projection structure of Photosystem II *in vivo* determined by cryo-electron crystallography. *Micron* 28: 439–446

Stoylova SS, Flint TD, Ford RC and Holzenburg A (2000) Structural analysis of Photosystem II in far-red-light-adapted thylakoid membranes – new crystal forms provide evidence for a dynamic reorganization of light-harvesting antennae subunits. *Eur J Biochem* 267: 207–215

Swiatek M, Kuras R, Sokolenko A, Higgs D, Olive J, Cinque G, Muller B, Eichacker LA, Stern DB, Bassi R, Herrmann RG and Wollman FA (2001) The chloroplast gene *ycf9* encodes a Photosystem II (PS II) core subunit, PsbZ, that participates in PS II supramolecular architecture. *Plant Cell* 13: 1347–1367

Trissl HW and Wilhelm C (1993) Why do thylakoid membranes from higher plants form grana stacks? *Trends Biochem Sci* 18: 415–419

Tsiotis G, McDermott G and Ghanotakis D (1996a) Progress towards structural elucidation of Photosystem II. *Photosynth Res* 50: 93–101

Tsiotis G, Walz T, Spyridaki A, Lustig A, Engel A and Ghanotakis D (1996b) Tubular crystals of a Photosystem II core complex. *J Mol Biol* 259: 241–248

Tsiotis G, Psylinakis M, Woplensinger B, Lustig A, Engel A and Ghanotakis D (1999) Investigation of the structure of spinach Photosystem II reaction center complex. *Eur J Biochem* 259: 320–324

Tsvetkova NM, Brain APR and Quinn PJ (1994) Structural characteristic of thylakoid membranes of *Arabidopsis* mutants deficient in lipid fatty-acid desaturation. *Biochim Biophys Acta* 1192: 263–271

Tsvetkova NM, Apostolova EL, Brain APR, Williams WP and Quinn PJ (1995) Factors influencing PS II particle array formation in *Arabidopsis thaliana* chloroplasts and the relationship of such arrays to the thermostability of PS II. *Biochim Biophys Acta* 1228: 201–210

Vácha F, Vácha M, Bumba L, Hashizume K and Tani T (2000) Inner structure of intact chloroplasts observed by a low temperature laser scanning microscope. *Photosynthetica* 38: 493–496

Vallon O, Wollman FA and Olive J (1985) Distribution of intrinsic and extrinsic subunits of PS II protein complex between appressed and non-appressed regions of the thylakoid membrane: an immunocytochemical study. *FEBS Lett* 183: 245–250

Vallon O, Wollman FA and Olive J (1986) Lateral distribution of the main protein complexes of the photosynthetic apparatus in *Chlamydomonas reinhardtii* and in spinach: an immunocytochemical study using intact thylakoid membranes and a PS II-enriched membrane preparation. *Photobiochem Photobiophys* 12: 203–220

van der Staay GWM and Staehelin LA (1994) Biochemical characterization of protein composition and protein phosphorylation patterns in stacked and unstacked thylakoid membranes of the prochlorophyte *Prochlorothrix hollandica*. *J Biol Chem* 269: 24834–24844

van Roon H, van Breemen JFL, de Weerd FL, Dekker JP and Boekema EJ (2000) Solubilization of green plant thylakoid membranes with *n*-dodecyl- α -D-maltoside. Implications for the structural organization of the Photosystem II, Photosystem I, ATP synthase and cytochrome *b6/f* complexes. *Photosynth Res* 64: 155–166

Vermaas WFJ (1994) Molecular-genetic approaches to study photosynthetic and respiratory electron transport in thylakoids from cyanobacteria. *Biochim Biophys Acta* 1187: 181–186

Whitmarsh J and Govindjee (1999) The photosynthetic process. In: Singhal GS, Renger G, Sopory SK, Irrgang KD and Govindjee (eds) Concepts in Photobiology: Photosynthesis and Photomorphogenesis, pp 11–51. Narosa Publishing House, New Delhi, India

Wollman FA, Olive J, Bennoun P and Recouvreur M (1980) Organization of the Photosystem II centers and their associated antennae in the thylakoid membranes: a comparative ultrastructural, biochemical and biophysical study of *Chlamydomonas* wild type and mutants lacking in Photosystem II reaction centers. *J Cell Biol* 87: 728–735

Xu Q and Bricker TM (1992) Structural organization of proteins on the oxidizing side of Photosystem II – two molecules of the 33 kDa manganese-stabilizing proteins per reaction center. *J Biol Chem* 267: 25816–25821

Yakushevska AE, Jensen PE, Keegstra W, van Roon H, Scheller HV, Boekema EJ and Dekker JP (2001) Supramolecular organization of Photosystem II and its associated light-harvesting antenna in *Arabidopsis thaliana*. *Eur J Biochem* 268: 6020–6028

Yamamoto Y, Ueda T, Shinkai H and Nishimura M (1982) Preparation of O₂-evolving Photosystem II subchloroplasts from spinach. *Biochim Biophys Acta* 679: 347–350

Zouni A, Witt HT, Kern J, Fromme P, Krauss N, Saenger W and Orth P (2001) Crystal structure of Photosystem II from *Synechococcus elongatus* at 3.8 Å resolution. *Nature* 409: 739–743