

Photosystem II: a multisubunit membrane protein that oxidises water

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A structure of photosystem II recently determined by X-ray crystallography at 3.8 Å resolution complements structural studies using high-resolution electron microscopy and represents a major step towards understanding how photosynthetic organisms use light energy to oxidise water.

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Abbreviations

Chl	chlorophyll
cyt	cytochrome
EM	electron microscopy
Pheo	pheophytin
PS	photosystem
RC	reaction centre

Introduction

At the heart of the photosynthetic process is photosystem (PS) II. This multisubunit complex is embedded in the thylakoid membranes of plants, algae and cyanobacteria, and catalyses the most thermodynamically demanding reaction in biology: namely, the splitting of water into dioxygen and reducing equivalents. The reaction is driven by solar energy and underpins the survival of essentially all life on our planet. It supplies the oxygen we breathe, it maintains the ozone layer needed to protect us from UV radiation and, of course, it supplies the reducing equivalents necessary to fix carbon dioxide to organic molecules, creating biomass, food and fuel.

Our understanding of the structure of the PSII complex has advanced dramatically over the past year or so, but before this structural work, a range of biochemical and biophysical techniques had provided a good understanding of the events that give rise to the primary and secondary electron transfer processes leading to water oxidation [1]. These processes are initiated by the absorption of light energy by the many chlorophyll and other pigment molecules associated with PSII. The nature of these PSII light-harvesting antenna systems varies under different growth conditions and with different types of organisms. However, within the PSII core complex, only chlorophyll (Chl) α and β -carotene are found, bound mainly to the CP43 and CP47 proteins. The excitation energy absorbed by these pigments is then transferred to the reaction centre (RC), composed of the D1 and D2 proteins.

Together, these RC proteins bind all the redox-active cofactors involved in the energy conversion process and the following sequence of reactions occurs. Firstly, a special form of Chl α , P680 (the primary electron donor of PSII), acts as an exciton trap and is converted to a strong reducing agent after excitation (P680*). Within a few picoseconds, P680* reduces a pheophytin (Pheo) molecule to form the radical pair state P680*+Pheo \bullet^- and, within a further few hundred picoseconds, Pheo \bullet^- reduces a plastoquinone molecule bound to the D2 protein (Q_A) to produce P680*+Pheo Q_A^- . The P680*, which has a very high redox potential (>1V), oxidises a tyrosine residue (Y_Z) of the D1 protein (Tyr161) to form $Y_Z^{\bullet+}$ +P680Pheo Q_A^- on a nanosecond timescale. On a millisecond timescale, Q_A^- reduces a second plastoquinone associated with the D1 protein (Q_B) to form $Y_Z^{\bullet+}$ +P680Pheo $Q_A Q_B^-$. The oxidised tyrosine radical ($Y_Z^{\bullet+}$) extracts an electron and a proton from a cluster of four manganese atoms that binds the two substrate water molecules. A second photochemical turnover reduces Q_B^- to Q_B^{2-} , which is then protonated and released from the D1 protein into the lipid bilayer to be subsequently oxidised by PSI via the cytochrome b_6f complex. Two further photochemical turnovers provide the manganese cluster with a total of four oxidising equivalents. Each oxidation state is represented in an S-state cycle shown and explained in Figure 1.

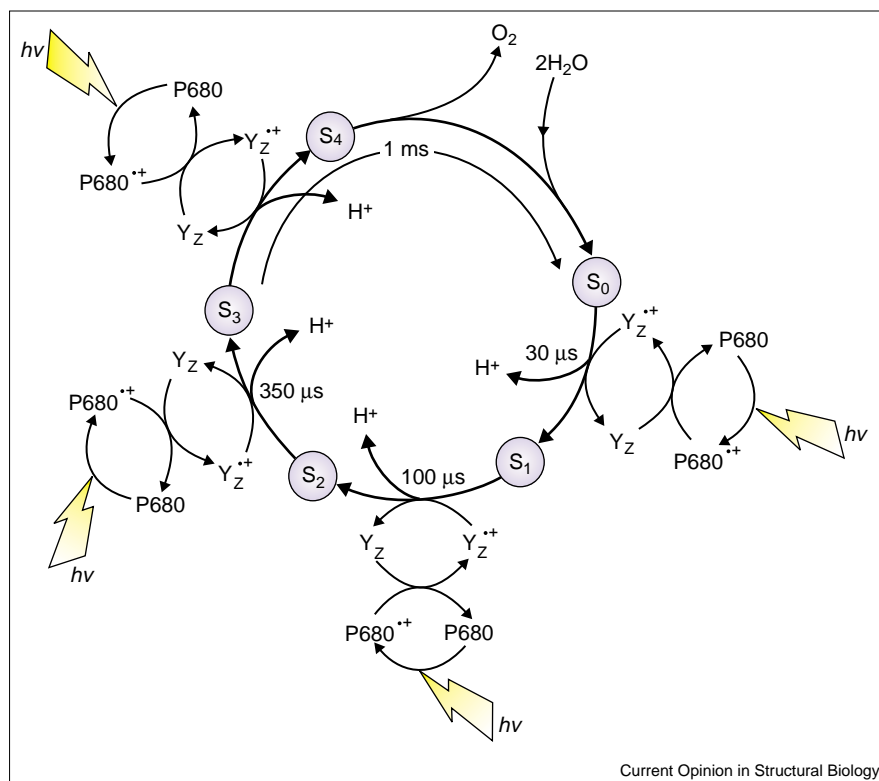
In addition to the above, some side reactions can occur under certain conditions, including the oxidation of a β -carotene molecule, a Chl α molecule and a high potential cytochrome (cyt b559) bound within the PSII core complex.

An essential prerequisite to understanding the above reactions in greater depth, particularly those involved in water oxidation, and possibly mimicking them in an artificial solar energy device is a detailed understanding of the three-dimensional (3D) structures of the participating macromolecular subunits. Both electron and X-ray crystallography have contributed to the goal of obtaining a full description of the molecular processes by which PSII functions as a light-driven water oxidase.

Organisation and structure of the protein subunits

On the basis of sequence analyses and hydropathy plots, it had been assumed that the D1 and D2 proteins of the PSII RC were structurally similar to the L and M subunits of the purple bacterial RC [2,3], for which an X-ray structure was determined more than 15 years ago [4]. This was confirmed in 1998 when the 3D structure of a subcomplex of PSII isolated from spinach was obtained by electron crystallography at a resolution of 8 Å [5,6]. Both the D1 and

Figure 1



The S-state cycle for the oxygen evolution reaction, as first suggested by Kok *et al.* [47], modified to accommodate the model proposed by Babcock and colleagues (reviewed in [37]). The scheme is based on the fact that, when a photosynthetic organism is exposed to a series of saturating flashes of light, the evolution of oxygen follows a period of four. This indicates that four oxidising equivalents must accumulate at a single catalytic centre before a dioxygen molecule is formed and released. Therefore, there must be four light-induced redox turnovers of PSII RC chlorophylls (P680) and tyrosine (Y_z) to produce the four oxidising equivalents accumulated in the Mn cluster. Dioxygen is produced on the S₄ to S₀ transition. According to the hypothesis of Babcock and colleagues, each photoinduced step of the S-state cycle (S₀ to S₄) involves the concerted removal of an electron and a proton from two bound water molecules. Other proposed mechanisms do not invoke a hydrogen abstraction at each step of the cycle [48,49].

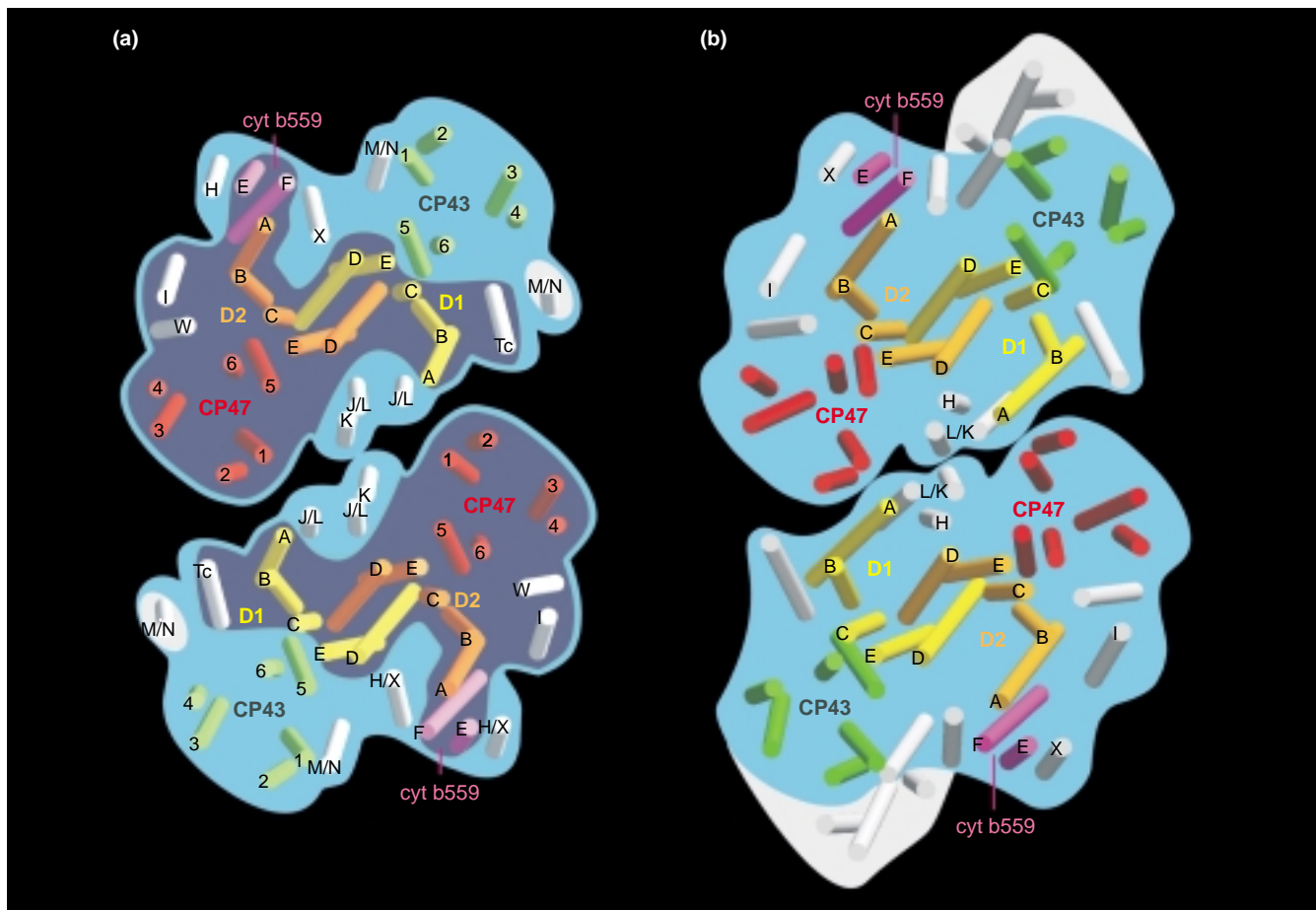
D2 subunits consist of five transmembrane helices related by a pseudo-twofold axis (shown in Figure 2a as yellow and orange cylinders for D1 and D2 proteins, respectively). It was also possible to assign six transmembrane helices to CP47, showing for the first time that these helices are arranged in a circular manner as three pairs (shown in Figure 2a as red cylinders). It was noted that this arrangement is similar to that of the six N-terminal transmembrane helices of the PSI RC proteins, which had been revealed by X-ray crystallography initially at 4 Å [7] but now at 2.5 Å resolution [8^{••},9^{••}]. Also assigned in the 3D map of PSII were seven other transmembrane helices, with two of them tentatively identified as the α and β subunits (PsbE and PsbF proteins, respectively) of cyt b559 [10] (shown in Figure 2a as magenta cylinders labelled E and F).

This electron crystallographic approach was extended to analyses of 2D crystals of the PSII core dimer complex isolated from spinach, leading to the publication of a projection map in 1999 [11] and a 3D structure in 2001 [12^{••}]. These studies provided the first direct evidence that the transmembrane helices of CP43 (shown in Figure 2a as green cylinders) are arranged in the same manner as those of CP47. Additionally, they were shown to be positioned on the opposite side of the RC proteins and are related to the CP47 helices by the same pseudo-twofold axis relating the D1 and D2 helices. In addition to the 22 transmembrane helices of the D1/D2/CP43/CP47 proteins,

each PSII monomer contains a further 10 transmembrane helices (shown as white cylinders in Figure 2a), excluding the two tentatively assigned to cyt b559. It was also concluded that two transmembrane helices, which were originally assigned to the CP47/D1/D2 subcomplex [6], were in fact the densities of two chlorophylls located towards the N termini of the D1 and D2 proteins. To date, this is the only 3D model of a higher plant PSII core complex at a resolution sufficient to reveal its transmembrane helices.

In 2001, an X-ray structure of PSII isolated from the thermophilic cyanobacterium *Synechococcus elongatus* was published at 3.8 Å resolution [13^{••}]. This work was consistent with the positioning of the major subunits within the higher plant PSII complex established by electron cryomicroscopy (cryo-EM), as indicated in Figure 2, and provided an interpretation of a recent 16 Å projection map derived from 2D crystals of the *S. elongatus* PSII core dimer complex [14[•]]. The X-ray structure of *S. elongatus* PSII also agreed with the organisation of transmembrane helices of these major subunits in the higher plant PSII structure derived from cryo-EM and unambiguously identified the position of the single transmembrane helices of the α and β subunits of cyt b559. As can be seen in Figure 2, the cryo-EM and X-ray analyses also broadly agreed on the positioning of nine other transmembrane helices, including a cluster of three at the monomer–monomer interface within the dimer.

Figure 2



Comparison of the transmembrane helix organisation of PSII core dimers from (a) higher plants (spinach) and (b) cyanobacteria (*S. elongatus*) derived from electron [12**] and X-ray [13**] crystallography, respectively. The transmembrane helices of D1 (yellow), D2 (orange), CP47 (red), CP43 (green) and cyt b559 (magenta) seem to be in identical positions. This is also true for nine of the single transmembrane helix low molecular weight subunits shown in white. However, the white background highlights differences between the two structural models: *S. elongatus* has three additional

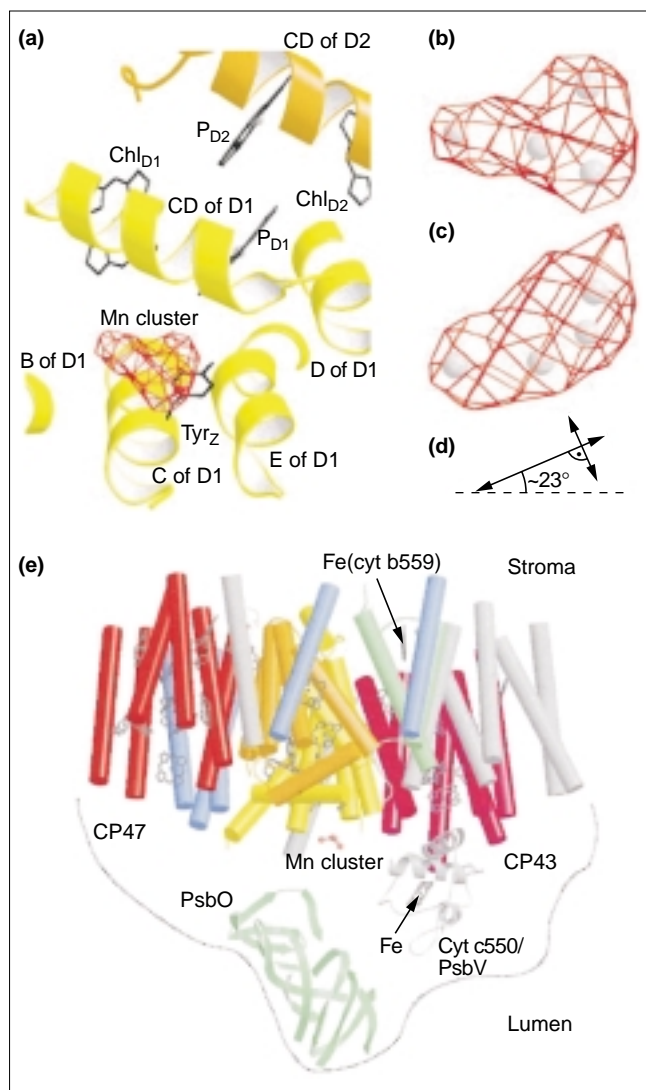
helices close to CP43, whereas the spinach structure suggests an additional transmembrane helix on the periphery of the complex on the D1/CP43 side. The dark blue region in (a) shows the helix organisation of the spinach CP47-RC subcomplex derived by Rhee *et al.* [6], but modified by the removal of two helices adjacent to helix B of the D1/D2 proteins that were assigned to densities that, in retrospect, were due to $\text{Chl}_{z,D1}$ and $\text{Chl}_{z,D2}$ (see Figure 4). The assignment of the small subunits is tentative in both structures, as described in [13**,16**]. Figure reproduced with permission from Hankamer *et al.* [17**].

However, there were some slight differences that may reflect variations between higher plant and cyanobacterial PSII, or may simply be due to different biochemical isolation procedures. The cyanobacterial structure has three additional helices close to CP43 compared with the spinach structure and an additional helix was found in the peripheral region in the vicinity of helix B of the D1 protein. Both structures have insufficient resolution to identify amino acid sidechains and the ordering of the helices of D1/D2/CP43/CP47 in Figure 2a relies on the known ordering of analogous helices in the purple bacterial and PSI RCs [4,8**]. The low resolution of the structural models has not allowed the unambiguous assignment of the additional single transmembrane helices to specific low molecular weight subunits (e.g. PsbH, PsbI, PsbJ, PsbK, PsbL, PsbM, PsbN, PsbT_c, PsbX and PsbZ) found

within the PSII complex [15,16**]. However, on the basis of other information, tentative assignments have been made, as shown in Figure 2, for both the higher plant [17**] and cyanobacterial [13**] systems.

As shown in Figure 3e, the X-ray analysis was able to reveal density for PsbV of *S. elongatus*, aided by the fact that this extrinsic protein is a cytochrome (cyt c550) containing a readily detectable haem iron. The extrinsic density in the X-ray map also contained a region of β strands, forming a tube 35 Å long with a diameter of about 15 Å tilted at 45° to the membrane plane (see Figure 3e). This feature has tentatively been assigned to about half of the manganese-stabilised protein PsbO, which is known to contain a considerable amount of β sheet [18,19,20*].

Figure 3



Location and orientation of the $(\text{Mn})_4$ cluster. (a) Close-up view of the PSII RC, with the electron density of the $(\text{Mn})_4$ cluster contoured at 5σ . The view is from the luminal side onto the membrane plane and shows that the $(\text{Mn})_4$ cluster is located at the luminal ends of the C and D helices of the D1 protein, and is close to the surface CD helix, as predicted by site-directed mutagenesis [25,26**]. (b) Enlarged view of the electron density of the manganese cluster. (c) View (b) rotated 90° around the horizontal axis (view along the membrane with the luminal side on top). (d) Orientation of the short and long axes of view (c). The latter is tilted 23° against the luminal side of the membrane plane (hatched line). (e) Side view of the PSII monomer looking down the long axis of the D1/D2 proteins, with a slightly tilted angle of the membrane plane. This view shows the luminal surface and the positioning of the Mn cluster, the PsbO protein assigned to the β -sheet structure (green) and cyt c550 (PsbV). The cylinders show the transmembrane helices of the various subunits within the monomer and can be compared with the luminal view shown in Figure 2b (note that the colour coding is slightly different). Figures reproduced with permission from Zouni *et al.* [13**].

Information on the structure and organisation of the extrinsic proteins of higher plants has been derived from single-particle analyses of the PSII supercomplex isolated from spinach [21,22,23**]. Difference mapping suggests

that a single copy of the PsbO protein lies along the luminal surface of the PSII core complex, stretching from the CP47 side across the luminal loops of the D1/D2 proteins to the region adjacent to CP43. A calculated remote model of the PsbO protein [20*] has been fitted into the available density and suggests the molecule is elongated, with a length in the region of 80 \AA and a width of about 15 \AA . The remaining extrinsic mass observed in the 3D model derived from single-particle analysis has been attributed to single copies of the PsbP and PsbQ extrinsic proteins, and to the luminal loops of the intrinsic RC core proteins, particularly the very large loops joining helices 5 and 6 of CP43 and CP47.

Organisation of chlorophylls and redox-active cofactors

The first direct structural information on the organisation of the PSII redox-active cofactors came from electron crystallography [6]. At 8 \AA resolution, it was possible to observe the densities of the porphyrin head groups of the bound chlorophylls. Within the D1/D2 helices, six densities were assigned, arranged around the twofold axis that related the D1 and D2 helices in a manner similar to that observed for cofactors within the purple bacterial RC. Importantly, however, it was noted that there was no 'special pair' (see below) and that the four chlorophylls on the electron donor side of the RC were equally spaced at about 11 \AA centre-to-centre. In the case of the two densities assigned to Pheo, however, their positioning seemed to match that of their counterparts in the bacterial RC. The CP47 subunit is a chlorophyll-binding protein and 14 densities within the electron density map were suggested to be due to the chlorophyll tetrapyrrole head groups. These chlorophylls were organised in layers towards the stromal and luminal surfaces. This organisation is consistent with many of the chlorophylls being ligated to histidine residues contained in the CP47 protein and predicted to be located at the ends of the transmembrane domains [24]. The X-ray diffraction analyses [13**] confirmed and extended these conclusions. The X-ray data not only gave more accurate information about the positioning and orientations of the four chlorophylls and two Pheo molecules clustered within the D and E transmembrane helices of the D1 and D2 proteins, but also confirmed the existence of two additional chlorophyll molecules, probably ligated to D1 His118 and D2 His117, which are usually referred to as Chl_{Z-D1} and Chl_{Z-D2} .

As Figures 3e and 4 show, the X-ray structure has also unambiguously located the haem irons of cyt b559 and cyt c550 (PsbV), as well as the non-haem iron located centrally between the Q_A^- and Q_B^- -binding sites on the D2 and D1 proteins, respectively. Densities were tentatively assigned to the Q_A plastoquinone and to D1 Tyr161 (Y_Z) and the twofold related D2 Tyr160 (Y_D). As in the case of earlier cryo-EM work, 14 chlorophylls were shown to be bound to CP47 and, in addition, 12 chlorophylls were identified within the six-helix bundle of CP43.

Perhaps the most important outcome of the X-ray diffraction analysis to date was the assignment of density to the manganese cluster $(\text{Mn})_4$ and the realisation that this density was located close to the surface helix on the luminal side joining transmembrane helices C and D of the D1 protein (see Figure 3a). This CD helix, as well as the C terminus of the D1 protein, was involved in the binding of the Mn cluster, as predicted by a wide range of experiments [1,25,26**]. Confirmation that the density was due to Mn was obtained by measuring X-ray edge anomalous diffraction. The density is shaped such that it can accommodate three Mn atoms at the corners of an isosceles triangle, with a fourth placed at the centre of the triangle (see Figure 3b). This arrangement and the interatomic distances are consistent with recent predictions arising from electron paramagnetic spectroscopy, ENDOR (electron nuclear double resonance) spectroscopy and X-ray spectroscopy [27*–29*].

Implications arising from the structural models

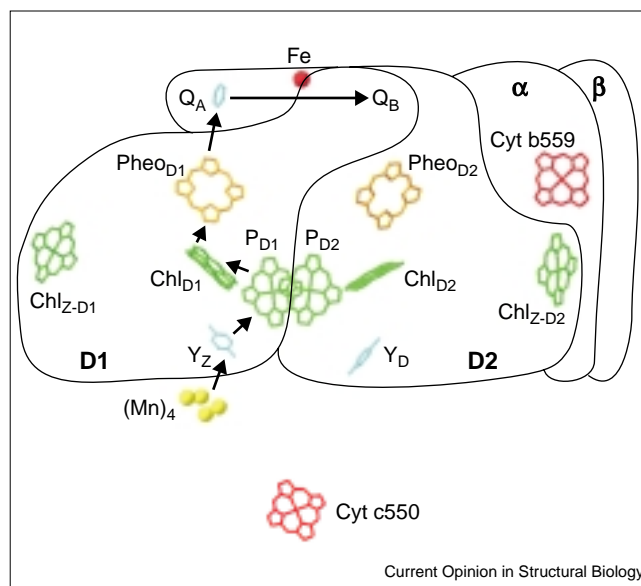
The main subunits of the PSII RC core are arranged such that the transmembrane helices of the CP47/D2 proteins are related to the transmembrane helices of the CP43/D1 proteins by a pseudo-twofold axis. This structural arrangement is similar to that found for the 11 transmembrane helices of the PSI RC proteins PsaA and PsaB [8**], indicative of a common evolutionary origin [6,30].

Although the main cofactors of the PSII RC are arranged around a twofold axis similar to that found in the purple bacterial RC (see Figure 3), P680 is not a 'special pair' of excitonically coupled chlorins, as found in the bacterial system or in the RC of PSI. This difference could be due to the requirement for P680 to generate a radical cation with a very high redox potential [31*,32].

The absence of a 'special pair' results in a shallow trap, such that PSII has a relatively high chlorophyll fluorescence yield compared with other types of photosystem. Moreover, delocalisation of the excited state of P680 (P680*) [33] suggests that primary charge separation could occur from the accessory chlorophyll (Chl_{D1}) [34], for which there is recent experimental evidence [35]. The primary radical cation ($\text{Chl}_{\text{D1}}^{\bullet+}$) might then induce oxidation of the P_{D1} or P_{D2} chlorophylls to form the radical cation $\text{P680}^{\bullet+}$, leading to electron abstraction from Y_{Z} or Y_{D} [31*,32,36**]. If true, this series of events makes PSII novel in terms of its primary electron transfer process compared with other types of photosystem. It also means that the four chlorophylls that make up the RC of PSII — Chl_{D1} , Chl_{D2} , P_{D1} and P_{D2} (see Figure 4) — are high potential species [31*].

In PSII, there is only one $(\text{Mn})_4$ cluster, positioned close to D1 Tyr161 (Y_{Z}). However, the distance of 7 Å between these two redox centres is relatively long, in order to accommodate a sequential e/H^+ abstraction from water

Figure 4



Organisation of the RC cofactors of PSII, derived by X-ray crystallography at 3.8 Å resolution [13**]. The arrows show the direction of electron transfer from the $(\text{Mn})_4$ cluster to the terminal plastoquinone acceptor Q_{B} . This quinone was not present in the 3D crystals analysed, but its position was inferred from the similarity between the structures of PSII and the purple bacterial RCs [2,3]. An outline of the D1, D2, α and β subunits has been drawn as a cartoon to emphasise each protein-cofactor relationship in a diagrammatic manner.

bound to the $(\text{Mn})_4$ cluster, as proposed by Babcock and colleagues (reviewed in [37]). Higher resolution structures are required to identify the ligands and protein environment of the $(\text{Mn})_4$ cluster in order to develop improved postulates for the water oxidation process and the related e/H^+ transfer reactions.

When the extrinsic proteins are present, the $(\text{Mn})_4$ cluster is buried within a protein matrix by about 30–40 Å from the luminal surface [13**,23**]. However, the precise role of this protein shield is not yet fully understood, although it is well established that the extrinsic proteins facilitate the availability of Ca^{2+} and Cl^- as cofactors for the water oxidation reaction [38].

Although positioned some way from the chlorophylls involved in primary charge separation (see Figure 4), $\text{Chl}_{\text{Z-D1}}$ and $\text{Chl}_{\text{Z-D2}}$ (about 25 Å) and cyt b559 (about 40 Å) can be photo-oxidised with millisecond kinetics under some conditions. According to the electron transfer theory, for these oxidations to occur in the millisecond time domain, intermediate electron carriers are needed. Over the past couple of years, this has stimulated a number of experiments, with the current view being that β -carotene molecules known to be bound to the D1/D2 proteins, but not seen in the 3.8 Å X-ray-derived model, act as the intermediate electron transport carriers. Whether electrons go from cyt b559 to P680 via $\text{Chl}_{\text{Z-D2}}$ or directly via β -carotene is a matter of intense debate at present [39,40*].

Cyt c550 (PsbV) is also positioned a long distance from P680 (about 28 Å), although, in this case, there is no report of this cytochrome undergoing PSII-driven photo-oxidation. Therefore, its function remains an enigma.

The high-resolution models are aiding the interpretation of 3D structures of larger PSII complexes that consist of the core dimer and light-harvesting complex (LHC) proteins. In particular, a LHCII-PSII supercomplex has been isolated from higher plants and green algae, and studied by EM and single-particle analysis [21,22,23**,41]. This work and related studies on even larger PSII complexes and arrays [42,43*] are helping to build a structural model of the complete PSII unit within natural photosynthetic membranes.

Perspectives

There have been considerable advances towards elucidating the structure of PSII in the past year or so. However, in many ways, the published X-ray work has not revealed too many unexpected features because previous spectroscopic and molecular biological analyses have, together with the analogy of the purple bacterial RC and cryo-EM work, provided a reasonably accurate structural model of PSII. What is required now is a higher resolution X-ray structure that will reveal amino acid sidechains and thus allow a detailed understanding of how the various cofactors and chlorophyll molecules within PSII relate with their protein environment. This information is needed particularly for the (Mn)₄ cluster, for which the ultimate goal will be to identify the substrate water molecules before and during their oxidation, so that the molecular basis of the S-state transitions can be understood. I have no doubt this information will soon be available.

In addition to the above challenge, there are other facets of PSII function that will also require a structural understanding. The rapid turnover of the D1 protein is truly a remarkable dynamic property of PSII, resulting from the toxic nature of the water-oxidising reaction [44]. As yet, the cryo-EM and X-ray structures do not help us to understand this unique property, other than the conclusion that CP43 lays adjacent to the D1 protein and that the former is displaced from the complex when D1 protein degradation and replacement occur [45,46**]. The bigger picture is also important because the isolated PSII core complexes used to obtain the high-resolution information normally have light-harvesting antennae systems associated with them when in the intact thylakoid membrane. In the case of *S. elongatus*, the light-harvesting system is composed of C-allophycocyanin and C-phycoerythrin, which together make up its phycobilisome. How and where the phycobilisome attaches and interacts with the core dimer in this, and other, cyanobacterium are yet to be determined. In the case of higher plants and green algae, the situation is much better. The structures of super-molecular complexes containing Chl_a/Chl_b-binding proteins have been investigated using EM and reasonable models have been achieved, aided by the incorporation of high-resolution structural data for their components [21,22,23**,41,42,43*].

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