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Minireview

Subunit positioning and transmembrane helix organisation in the core dimer of photosystem II

Ben Hankamer[a], Ed Morris[a], Jon Nield[a], Alex Carne[b], James Barber[a,*]

[a] *Wolfson Laboratories, Department of Biological Sciences, Imperial College of Science, Technology and Medicine, London SW7 2AY, UK*
[b] *Institute of Cancer Research, Chester Beatty Laboratories, London SW3 6JB, UK*

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Abstract Recently 3D structural models of the photosystem II (PSII) core dimer complexes of higher plants (spinach) and cyanobacteria (*Synechococcus elongatus*) have been derived by electron [Rhee et al. (1998) Nature 396, 283–286; Hankamer et al. (2001) J. Struct. Biol., in press] and X-ray [Zouni et al. (2001) Nature 409, 739–743] crystallography respectively. The intermediate resolutions of these structures do not allow direct identification of side chains and therefore many of the individual subunits within the structure are unassigned. Here we review the structure of the higher plant PSII core dimer and provide evidence for the tentative assignment of the low molecular weight subunits. In so doing we highlight the similarities and differences between the higher plant and cyanobacterial structures.

Key words: Photosynthesis; Photosystem II; Structure; Electron microscopy; X-ray diffraction; Low molecular weight subunit

1. Introduction

Photosystem II (PSII) is the multisubunit membrane protein complex embedded in the thylakoid membranes of higher plants, algae and cyanobacteria that drives the water oxidation reaction of photosynthesis. This reaction splits water into molecular oxygen, protons and electrons, thereby sustaining an aerobic atmosphere on Earth and providing the reducing equivalents required for carbon fixation on a global scale. The solar energy required to drive this highly oxidising reaction is captured by specialised pigment–protein antenna systems, designed to transfer excitation energy efficiently to photochemically active reaction centres (RC). Though similar in function, these antenna systems form a diverse range of structures, with those of higher plants and green algae being located in the thylakoid membrane while those of most classes of cyanobacteria are bound extrinsically to the stromal surface of PSII.

1.1. The antenna systems of higher plants

The higher plant outer antenna system (Fig. 1A) consists of six membrane proteins (Lhcb1–6) [4] all of which bind chlorophyll (chl) *a*, chl *b* and carotenoids. Similar chl *a*/chl *b* binding proteins also make up the outer antennae of green algae. The structures of two of the higher plant proteins, Lhcb1 and 2, which form a heterotrimer, were determined at a resolution of 3.4 Å by electron crystallography [5]. Each monomer was found to have three transmembrane helices and one surface helix. Seven chl *a*, six chl *b* and two carotenoid molecules were also identified in each monomer. Based on this structural information and the sequence homology between the six Lhcb proteins the remaining four are also predicted to have three transmembrane helices and to ligate pigments in a similar way. Another PSII protein, PsbS (coloured cyan in Fig. 1A) shares considerable homology with the Lhcb proteins, except that it is predicted to have four transmembrane helices [4].

The Lhcb proteins of the antenna dock with the hydrophobic edge of the PSII core to form an excitonically coupled pigment network across a range of higher order PSII–antenna complexes [6,7]. The first such high order complex to be isolated was a PSII–LHCII supercomplex from higher plants [8] and a similar complex was subsequently purified from green algae [9]. Both structures, which showed considerable homology, were determined at intermediate resolution by single particle analysis [9,10]. In these supercomplexes a central PSII core dimer is flanked by two clusters of Lhcb proteins which, in the case of higher plants, contain Lhcb1, 2, 4 and 5 [8]. Each cluster is composed of a LHCII trimer (Lhcb1 and 2) at its outermost tip, which appears to be structurally and excitonically coupled to the PSII core dimer via monomers of the Lhcb proteins 4 and 5 also known as CP29 and CP26, respectively. Lhcb4 and 5, together with Lhcb6 (CP24), are also thought to facilitate the binding of additional LHCII trimers, probably containing Lhcb3 as well as Lhcb1 and Lhcb2, to the edge of the supercomplex thus forming still higher order structures referred to as mega-complexes by Boekema et al. [7].

1.2. Cyanobacterial antenna systems

Most cyanobacteria have distinctly different pigment–protein antenna systems, referred to as phycobilisomes [11]. These hemidiscoidal structures consist of rod-like arrays of water-soluble phycobiliproteins, bound to the stromal surface of the PSII core by linker proteins (Fig. 1B). The rods are composed of discs that are hexamers of αβ monomers. Each αβ monomer covalently binds two (allophycocyanin) or three (phycocyanin) or more (phycoerythrin) open chain tetrapyrroles. Depending on specific organisms two, three or five allophycocyanin rods lie along the surface [12] of the PSII complex

*Corresponding author. Fax: (44)-20-7594 5267.
E-mail address: j.barber@ic.ac.uk (J. Barber).

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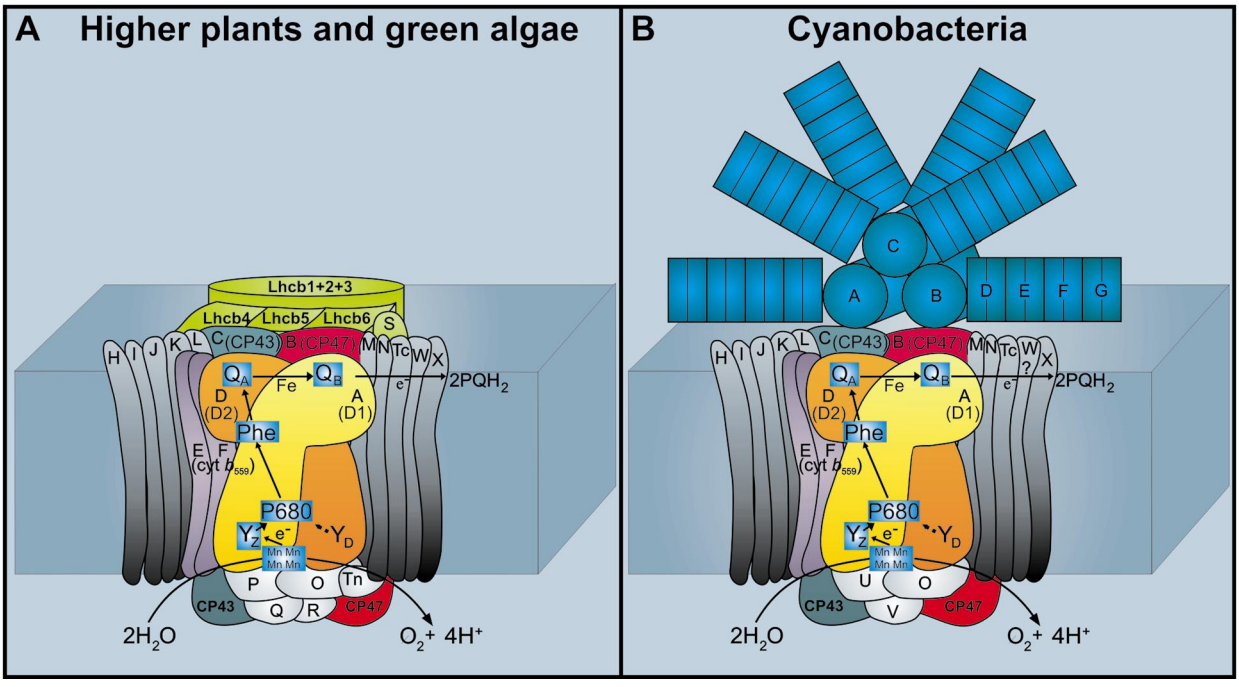

Fig. 1. Cartoon of the structure and subunit composition of PSII. A: Higher plants and green algae. B: Phycobilisome-containing cyanobacteria. The proteins of the core complex including the extrinsic proteins of the oxygen-evolving complex are labelled according to the gene nomenclature (e.g. PsbA = A) with common designations given for the major subunits (e.g. A = D1 protein). The outer light harvesting proteins are coloured light green for the plant systems (intrinsic Lhcb proteins) and blue-green for the cyanobacterial systems (extrinsic phycobiliproteins forming a phycobilisome where A, B and C are allophycocyanin rods) and D, E, F and G are discs of other phycobiliproteins (e.g. C-phycocyanin). The electron transfer pathway from water oxidation to plastoquinone reduction (PQH_2) is shown (see text).

while the phycocyanin and phycoerythrin rods radiate out as shown in Fig. 1B. The structures of a number of phycobiliproteins have been determined to better than 2.5 Å by X-ray crystallography [11] and provide important information about the binding sites, orientation and spatial relationships of the pigments. Although models have been proposed [13], the precise nature of how these basic structural units interact with one another to form the macroscopic antenna systems within the intact organism is not fully understood and neither are the details of their structural linkage with the PSII core.

1.3. The redox active components of the PSII core

The PSII core is the minimal unit which is capable of catalysing full PSII function common to all higher plants, algae and cyanobacteria. For reasons not fully understood, this complex exists as a dimer within the thylakoid membrane. Within each monomer, light-induced charge separation occurs between the lumenal and the stromal sides (Fig. 1). The primary electron donor, P680, a chl *a* molecule, donates an electron to the primary electron acceptor, pheophytin (Phe). Electron flow proceeds from Pheo to a plastoquinone molecule, Q_A, and subsequently to a second plastoquinone molecule, Q_B. The Q_A plastoquinone is a one-electron acceptor and is firmly bound in the Q_A binding pocket. In contrast, the Q_B plastoquinone is released from its binding site upon receiving two electrons from Q_A and two protons from the

Table 1
LMW proteins of PSII

Protein	Subunit	Mass (kDa)	Conserved amino acids in higher plants (%)	Special features
PsbE (c)	α-cyt b_{559}	9.255 (S)	62	Long lumenal C-terminal extension
PsbF (c)	β-cyt b_{559}	4.409 (S)	58	Short lumenal C-terminal extension
PsbH (c)	H protein	7.697 (S)	30	Long N-terminal extension/phosphorylation
PsbI (c)	I protein	4.195 (S)	53	Short stromal N-terminal extension
PsbJ (c)	J protein	4.116 (S)	75	Highly conserved sequence
PsbK (c)	K protein	4.283 (S)	59	Short stromal C-terminal extension
PsbL (c)	L protein	4.366 (S)	73	Highly conserved sequence
PsbM (c)	M protein	3.755 (P)	41	Short N-terminal extension
PsbN (c)	N protein	4.722 (T)	33	Short N-terminal extension
PsbT (c)	T_c protein	3.849 (S)	71	Short N-terminal extension
PsbW (n)	W protein	5.928 (S)	48	Lumenal N-terminus
PsbX (n)	X protein	4.225 (S)	–	

These proteins are products of the *psb*E to *psb*X genes that occur in all types of oxygenic organisms except for possibly PsbW. In eukaryotic organisms the *psb* genes are located in either the chloroplast (c) or nuclear (n) genes. The molecular masses of the mature PsbE–PsbX proteins are calculated from the protein sequences reported in the SwissProt database using the MacBioSpec program (Sciex Corp., Thornhill, ON, Canada) for spinach (S), pea (P) and tobacco (T). The percentage of conserved amino acids in higher plants is based on the mature protein where available.

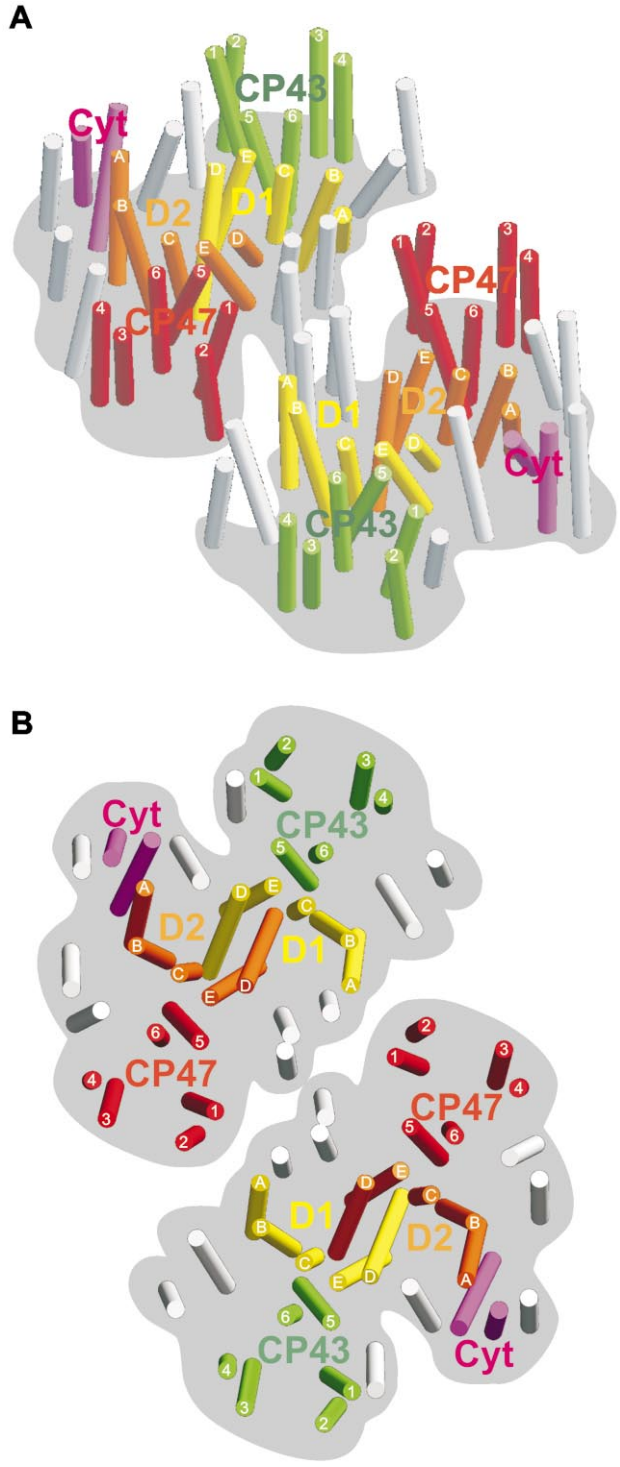

Fig. 2. 3D structural model of PSII core dimer of spinach derived by electron crystallography [2] showing (A) oblique view and (B) top view from the lumen. Colour coding of major subunits corresponds with that used in Fig. 1.

stroma. On the lumenal side, the oxidised form of P680 is reduced by a redox active tyrosine, which in turn is reduced by a Mn ion contained within a cluster of four. When the $(Mn)_4$ cluster accumulates four oxidising equivalents, two water molecules are oxidised to yield dioxygen and four protons.

1.4. The PSII core subunits

The PSII core consists of over 20 subunits. All the redox active cofactors involved in the water/plastoquinone activity of PSII are bound to the RC proteins, D1 and D2. Closely associated with these two proteins are the chl *a* binding inner antenna proteins, CP43 and CP47, and the extrinsic lumenally bound proteins of the oxygen-evolving complex (OEC). The OEC of higher plants and green algae consists of the 33 kDa PsbO, 23 kDa PsbP and 17 kDa PsbQ subunits, while in cyanobacteria PsbP and PsbQ are replaced by 9 kDa PsbU and 13 kDa PsbV (cytochrome c_{550}) proteins. Higher plants and green algae are reported to bind two additional extrinsic proteins on the lumenal surface, PsbR and $PsbT_n$ [14]. Over and above these subunits, the PSII core complexes of higher plants, green algae and cyanobacteria all contain a large number of low molecular weight (LMW) intrinsic proteins (Table 1), including the α- and β-subunits of cytochrome b_{559} (cyt b_{559}).

Electron crystallography of higher plant (Fig. 2) [1,2,15] and X-ray crystallography of cyanobacterial [3] PSII have revealed the locations of the main subunits and the relative positioning of their transmembrane helices. These studies have also given important information about the organisation of the redox active cofactors and chl *a* molecules bound within the D1, D2, CP43 and CP47 subunits.

2. Transmembrane helix organisation of major subunits

In 1996 we grew 2D crystals of a spinach PSII subcomplex (CP47–RC) composed of the D1, D2, CP47, cyt b_{559} proteins

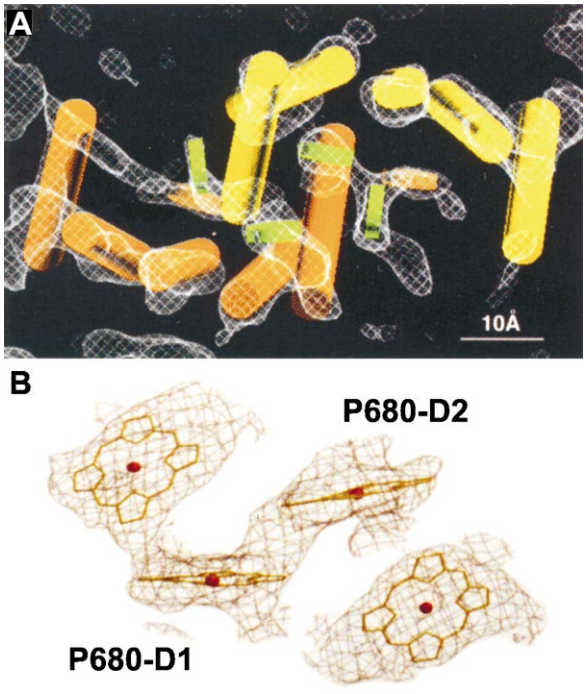

Fig. 3. A: Positioning of the four core chlorophylls (green) within the D1 (yellow) and D2 (orange) transmembrane helices showing the absence of a special pair. The data were obtained by electron crystallography and published in Rhee et al. [1]. B: Positioning of the four core chlorophylls of P680 as determined by X-ray crystallography confirming the absence of a special pair in PSII [3]. The labelling emphasises the P680 chlorophylls on the D1 and D2 sides of the RC.

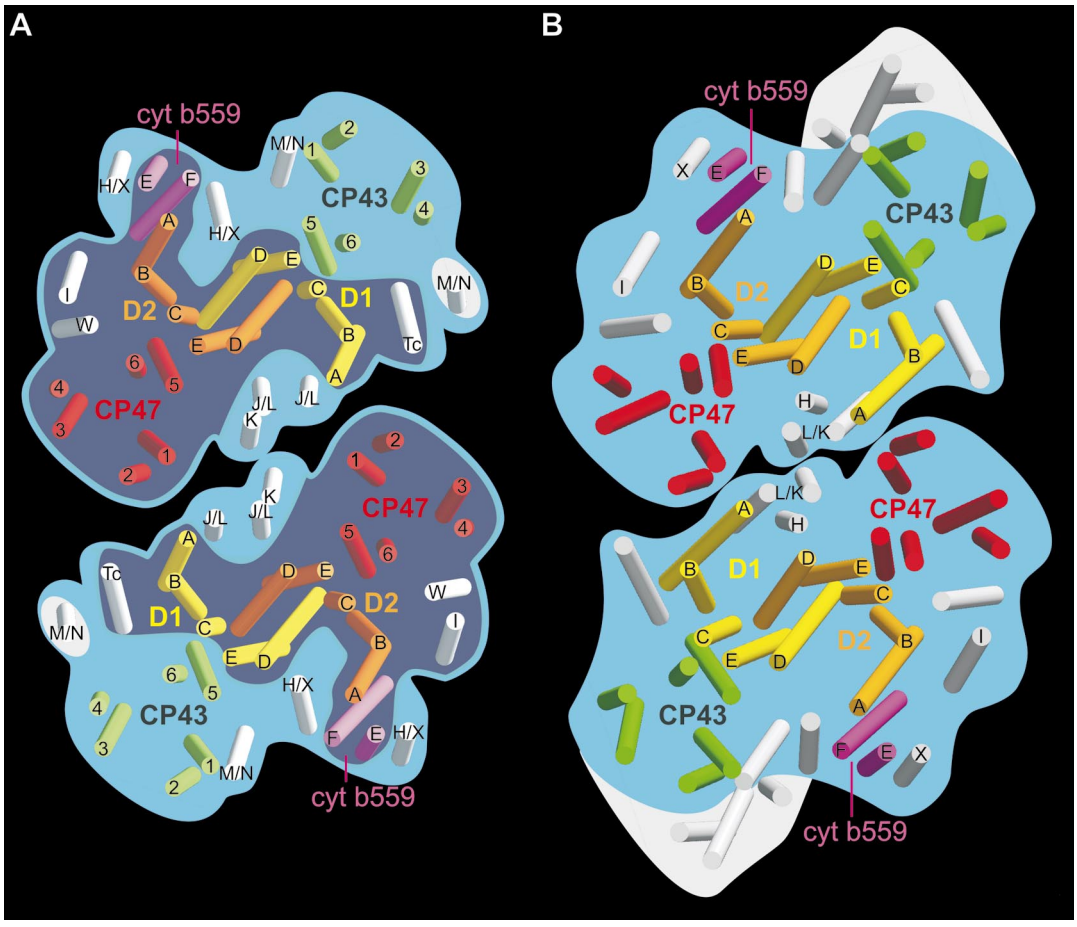

Fig. 4. Assignment of the LMW single transmembrane helical subunits in the PSII core dimer of (A) spinach and (B) *S. elongatus* where the latter is taken from Zouni et al. [3]. The dark blue region in A shows the helix organisation of the spinach CP47–RC subcomplex derived from Rhee et al. [1] modified by removal of the two transmembrane helices adjacent to helix B of the D1 and D2 proteins (see text). The remaining cyan regions contain the additional transmembrane helices of CP43 and other LMW subunits present in the core dimer. The colour coding is as in Figs. 1 and 2.

and a number of LMW subunits. Analysis of these 2D crystals by cryo-electron microscopy (cryo-EM) was carried out in collaboration with Werner Kühlbrandt and Kyong-He Rhee and an 8 Å projection map was published in 1997 [16] followed by a 3D structure in 1998 [1]. This work revealed for the first time the organisation of the transmembrane helices of the D1, D2 and CP47 proteins (see Figs. 2 and 4A). The two sets of five transmembrane helices of the D1 and D2 proteins were shown to be arranged around a pseudo-two-fold axis in a conformation similar to that of the L- and M-subunits of the RC of purple bacteria [17] and consistent with predictions based on sequence homology studies [18]. The CP47 subunit was found to consist of six transmembrane helices arranged in a ring of three pairs located adjacent to helices C and E of the D2 protein. This three helix pair motif is remarkably similar to that of the inner antenna domain of PSI corresponding to the six N-terminal helices of the PsaA or PsaB RC proteins [19]. Furthermore it was noted that the overall arrangement of the 16 helices of CP47, D1 and D2 proteins resembles the organisation of 16 of the 22 transmembrane helices of the PsaA/PsaB RC proteins of PSI. This similarity provided direct evidence for the proposed structural homology and common evolutionary origins of PSI and PSII [20]. Within the CP47–RC structure, seven further protein densities were attributed to single transmembrane helices and two of these were assigned to the α- and β-subunits of cyt b_{559} [1,21].

As the CP47–RC subcomplex does not drive the water oxidising reaction, methodologies were developed to grow 2D crystals of oxygen-evolving PSII core dimers isolated from spinach. The first crystals were analysed after negative staining and a 3D model constructed at a resolution of about 30 Å [22]. Since then the 2D crystals have been improved and subjected to cryo-EM. The resulting projection map at 9 Å was published in [15]. At this resolution the detailed density distribution corresponding to two sets of the CP47–RC subcomplex were clearly recognised within the dimer and new densities corresponding to additional subunits were also identified. Consequently, the location of CP43 on the opposite side of the D1/D2 heterodimer to that of CP47 was deduced (see Figs. 2 and 4A). Indeed, the density assigned to CP43 indicated that its six transmembrane helices were organised in the same way as those of CP47 and related to each by the pseudo-two-fold axis of the D1 and D2 heterodimer, like their PsaA/PsaB counterparts in PSI.

Among the new densities identified in the 9 Å projection map core dimer was a central region at the interface between the two monomers, a density close to helix B of the D1 protein and another in the region adjacent to helices 1 and 2 of

CP43. These additional features have now been resolved into transmembrane helices by merging tilt data to obtain a 3D map at about 10 Å resolution [2]. The new 3D map of the spinach PSII core dimer gave rise to the model shown in Fig. 2 in oblique (A) and top (B) views. The map confirmed the positioning of the six transmembrane helices of CP43 and revealed additional helices belonging to LMW subunits (see below).

The overall organisation of the transmembrane helices of the major subunits D1, D2, CP43 and CP47 in the new 3D map confirmed our earlier conclusions [15] and emphasised the structural similarity of the helix organisation of the D1/CP43 and D2/CP47 clusters with that of the PsaA and PsaB RC proteins of PSI [20]. The ordering of the helices in Fig. 2 is based on the structural analogies of the D1 and D2 proteins with the L- and M-subunits of the purple bacterial RC [23] and those of CP43 and CP47 with the N-terminal helices of the PSI RC [24]. Although the comparison of the CP47–RC subcomplex and the PSII core dimer complex unambiguously identified the densities of CP43 and CP47 (see Fig. 4A), the electron crystallographic derived models could not distinguish between the D1 and D2 proteins. The suggestion that the D1 protein is located adjacent to CP43 was initially based on the work of Barbato et al. [25], which indicated that the rapid turnover of the D1 protein due to photoinhibitory damage involves the displacement of CP43 from the core complex. In support of this assignment, light-induced cross-linking studies showed a close interaction between D1 and CP43 [26]. The recent structure of PSII from *Synechococcus elongatus* confirmed this assignment by locating the $(Mn)_4$ cluster in the X-ray derived map. It is known that the $(Mn)_4$ cluster must be close to Tyr-161 of the D1 protein [27] and thus the transmembrane helices of the two RC proteins are now unambiguously defined.

3. Cofactors and chlorophylls

The PSII core dimer structure of spinach modelled in Fig. 2 has a resolution of about 10 Å [2], but the earlier structure of the CP47–RC complex [1] was at 8 Å, a resolution sufficient to differentiate densities which could be attributed to chl molecules. This led to the assignment of six porphyrins within the four helical cluster consisting of the D and E transmembrane helices of the D1 and D2 proteins. These were organised around a two-fold-axis in a way very similar to that of the cofactors within the RC of purple bacteria [1]. Thus two densities were assigned to Pheo molecules and the remaining four, located towards the lumenal side of the helical bundle, to chl *a*. However, unlike the bacterial RC, no 'special pair' of chls was found. Instead the four chls were equally spaced with centre-to-centre distances of about 10–11 Å (see Fig. 3A). In the absence of a special pair no strong excitonic coupling occurs, making P680 a shallow trap compared with its bacterial counterparts. Therefore P680 can be considered a tetramer of chls with similar singlet state energies and redox potentials. Confirmation of this arrangement has now come from the X-ray diffraction analysis of the cyanobacterial RC (see Fig. 3B) [3] and its implications have been discussed in a recent paper [28]. The X-ray structure showed that two chl molecules, equivalent to the accessory bacteriochlorophylls of the bacterial RC, are tilted at about 30° to the membrane plane while the chls corresponding to the special pair are positioned vertical to the membrane plane [3]. As found by electron crystallography, the centre-to-centre distances for the four chls is about 10 Å. The monomeric nature of the chls which constitute P680 is probably needed in order for this primary donor to generate the high redox potential necessary to drive the water splitting process. Two additional chl molecules were predicted to ligate to the D1/D2 heterodimer via His118 located in the B-helix of both proteins [29]. The densities due to these two chls were probably mistaken as transmembrane helices in the CP47–RC electron crystallographic map [1]. However, the existence of these two peripheral chls was confirmed by the X-ray work [3]. No equivalent chls are found in the peripheral regions of the L- and M-subunits of the bacterial RC and their precise function in PSII has yet to be elucidated.

The 8 Å map of CP47–RC [1] revealed 14 densities within the six helical bundle of transmembrane helices assigned to CP47 which were attributed to chl molecules. These chls were located towards the stromal and lumenal surfaces. The X-ray structure [3] confirmed this general arrangement within CP47 and identified the position and approximate orientations of 13 chls. Similarly, 11 chls were assigned to CP43 and again these tended to be layered towards the stromal and granal surfaces of the six transmembrane helical bundle [3].

4. LMW subunits

Although the electron and X-ray crystallographic studies have revealed the organisation of the transmembrane helices within PSII core dimers of spinach [1,2] and *S. elongatus* [3], respectively, they are at insufficient resolution to trace side chains. Therefore the identification of specific helices of the major subunits, D1, D2, CP43 and CP47, relies on comparison with the high resolution structures of the RCs of purple photosynthetic bacteria [23] and PSI [24]. In the case of the LMW subunits, no homologies can be drawn with other types of RCs and it therefore remains a challenge to assign specific proteins to particular densities.

Table 1 gives a list of LMW proteins of PSII and below the properties of each is briefly described. These descriptions of the LMW subunits of PSII complement and update an earlier review where additional primary references can be found [14]. The nomenclature used is based on the names of the genes which encode them where '*psb*' stands for 'photosystem b'. *psb*A, *psb*B, *psb*C and *psb*D encode the major subunits, the D1, CP47, CP43 and D2 proteins, respectively. The remaining subunits are denoted Psb proteins.

4.1. PsbE and PsbF

These are the α- and β-subunits respectively of cyt b_{559}. After processing, the PsbE and PsbF contain 82 and 38 amino acids in most higher plants and have molecular masses of about 9.3 and 4.4 kDa [30]. Both subunits have a single transmembrane helix with their N-termini exposed to the stromal surface [31].

The α-subunit is characterised by having a long C-terminus of about 44 amino acid residues extending from the lumenal surface of the membrane while the β-subunit has essentially no lumenal domain. Of considerable importance is that each subunit contains conserved histidine residues which are located within the membrane-spanning region towards the stromal surface. Electron paramagnetic resonance measurements

predicted that these histidines form axial ligands to the haem of the cytochrome [32]. Helices coloured purple in Fig. 2 were suggested to be those of cyt b_{559} based on electron crystallography [21] and this was confirmed by X-ray analysis [3]. Although it had been argued by some that there are two cyt b_{559} per RC [33] there is evidence favouring a single copy of the cytochrome per RC [34]. The recent structural studies support the latter stoichiometry. The deletion of the *psb*E gene results in a lack of assembly of PSII [35]. On the other hand, modification of the His residue in the α-subunit can lead to an assembled and functionally active PSII complex even when no haem is ligated to the PsbE and PsbF proteins [36]. Normally the haem has a high redox potential of about 0.4 V but under some circumstances this potential is lowered to 0.2 V or less. The chemical basis of this redox shift and its significance, if any, is unclear. Under some circumstances, usually when the water splitting reactions are inhibited such as at low temperature, the haem of cyt b_{559} is oxidised by $P680^+$. According to the recent structural models [1–3], the haem is at least 40 Å from P680, which is too far to account for the millisecond time constant for its oxidation. Accordingly it has been suggested that a β-carotene molecule bound within the D1/D2 heterodimer acts as a redox intermediate between the cyt b_{559} and P680 [37]. The reduction of the oxidised haem may involve Q_A^-, Q_B^- or $Pheo^-$ [38] but again there is a distance problem, with the required electron donation occurring over distances of 28 Å or more according to the recent X-ray structure [3].

The physiological role of cyt b_{559} remains a matter for debate but based on the recent work of Morais et al. [36] it is clearly not involved in water oxidation. It seems likely that cyt b_{559} plays a protective role in minimising photodamage of the RC [38].

4.2. PsbG

A chloroplast gene was initially called *psb*G by Steinmetz et al. [39] but it was shown later by Nixon et al. [40] that the product of this gene was not a PSII protein but rather a component of a chloroplast located NADPH/quinone oxidoreductase. This gene is now known as *ndh*K.

4.3. PsbH

In higher plants, the PsbH protein contains 72 amino acids and has a calculated mass of about 7.7 kDa. In cyanobacteria the protein is slightly smaller (~6.5 kDa) with a truncated N-terminus and as such does not have the N-terminal threonine residue, that is conserved in all higher plant and green algal sequences and is the site for reversible phosphorylation [41]. A report that the cyanobacterial PsbH can also undergo reversible phosphorylation [42] has not been confirmed. The functional significance of the phosphorylation process is unclear. The higher plant protein is characterised by having a single transmembrane helix with a long N-terminal region consisting of about 41 amino acids at the stromal surface, which reduces to about 30 residues in the case of cyanobacteria. The *psb*H gene can be deleted from the cyanobacterium *Synechocystis* without impairing the assembly of PSII and photoautotrophic growth [43]. The knockout mutant was, however, more sensitive to photoinhibition than the wild type. This sensitivity seemed to be due to the partial inhibition of the D1 protein repair process rather than to an increase in photochemical damage [44]. In contrast, when the *psb*H gene was deleted in *Chlamydomonas*, PSII did not assemble [45].

It has been suggested that the PsbH protein is involved in regulating Q_A to Q_B electron transfer and in this way is comparable with the H-subunit of the purple bacterial RC [46]. This suggestion, together with hints that PsbH stabilises the dimeric form of the PSII RC core complex [47], may have led Zouni et al. [3] to place PsbH close to helices D and E of the D2 protein at the monomer–monomer interface within the cyanobacterial core dimer (see Fig. 4B).

4.4. PsbI

The PsbI protein has a molecular mass of about 4.2 kDa. In most eukaryotes it contains 35 amino acids and is predicted to have a single transmembrane helix with a short N-terminal region at its stromal end. Since it is present in the isolated D1/D2 cyt b_{559} complex [48] it must be located close to the RC heterodimer. Indeed, there are reports that it can be chemically cross-linked with both the D2 protein and the α-subunit of cyt b_{559} [49]. Interestingly, the mature protein retains the initiating N-formyl group at its N-terminal methionine residue [30]. The *psb*I gene has been deleted in *Chlamydomonas* [50] and *Synechocystis* [51] without inhibiting PSII assembly and photoautotrophic growth. Thus the function of the PsbI protein remains unknown.

4.5. PsbJ

In most organisms the *psb*J gene is located in a gene cluster also containing the *psb*E, *psb*F and *psb*L genes [52]. The PsbJ protein is highly conserved, consists of 39 amino acids and has a calculated molecular mass of 4.1 kDa. It is predicted to have one transmembrane helix. The *psb*J gene has been deleted in *Synechocystis* [53] to generate a knock-out mutant which assembled PSII at a lower level than the wild type and consequently had a slower photoautotrophic growth rate.

4.6. PsbK

This ~4.3 kDa protein is highly conserved and has 37 amino acid residues. It is predicted to have a single transmembrane helix. Of particular note is that in higher plants, 24 amino acids are post-translationally cleaved from the initial gene product. In cyanobacteria eight amino acids are removed after translation. These pre-sequences probably bring about the insertion of the protein into the thylakoid membrane such that its N-terminus is on the lumenal side.

Deletion of the *psb*K gene in *Synechocystis* had very little effect on photoautotrophic growth and PSII activity [54,55]. In the case of *Chlamydomonas*, however, the deletion of the gene destabilised the PSII complex and the transformant was unable to grow photoautotrophically [56].

4.7. PsbL

This highly conserved PSII protein contains 37 amino acids and has a calculated molecular mass of about 4.4 kDa [57]. In most organisms its gene is located in the cluster together with *psb*E, *psb*F and *psb*J [58]. Hydropathy analysis suggests that it contains a single transmembrane helix. Inactivation of the *psb*L gene in *Synechocystis* resulted in a loss of PSII-mediated oxygen evolution and the transformant was unable to grow photoautotrophically [58]. It has also been reported that PsbL is required for normal functioning of the Q_A site based on

studies with isolated PSII complexes [59]. This conclusion was reinforced by the finding that PsbL is required for the oxidation of Tyr_z by $P680^+$ [60]. As a consequence the primary quinone acceptor is destabilised by the increased probability of rapid recombination between Q_A^- and $P680^+$.

4.8. PsbM

The PsbM protein is predicted to have one transmembrane helix with a very short N-terminal extension on the stromal side. It contains 33 amino acids and has a molecular mass of about 3.7 kDa. PsbM has been identified in PSII isolated from cyanobacteria [61] and the green alga *Chlamydomonas* [62].

4.9. PsbN

As in the case of PsbM, this protein is predicted to be in both cyanobacterial and plant PSII. However, it has not been detected directly in plant PSII although it has been identified in cyanobacteria [61]. It has a predicted single transmembrane helix. Deletion of both *psb*N and *psb*H genes from *Synechocystis* caused no effect other than those observed in the absence of *psb*H alone [43]. Thus the function and location of *psb*N are unknown.

4.10. PsbO, PsbP, PsbQ, PsbR and $PsbT_n$

These are all extrinsic proteins of the OEC complex and have no transmembrane helices. They therefore are not considered here in detail, but see [14].

4.11. PsbS

This is a peripheral 'CAB-like' protein of higher plants with four transmembrane helices [4]. It is not present in the PSII–LHCII supercomplex [63] and therefore is not a component of the PSII core complexes for which there are high resolution structural models.

4.12. $PsbT_c$

The chloroplast-encoded $PsbT_c$ protein is also found in cyanobacteria. It contains 30–34 amino acids, has a molecular weight of about 3.9 kDa and retains the initiating N-formyl group on its N-terminal methionine residue [64]. It is predicted to have a single transmembrane helix which is located at the N-terminus of the protein. The *psb*T_c gene was formerly known as ycf8 and is located close to the *psb*B gene in the chloroplast genome. The first identification of this subunit was made by comparing wild type and a mutant of *Chlamydomonas* that lacked the *psb*T_c gene [65]. This work also showed that the $PsbT_c$ is a PSII protein and is required for optimal activity under high light conditions so as to prevent photoinhibition.

4.13. PsbW

The PsbW protein is found in higher plants and green algae. A cyanobacterial 'PsbW-like' gene has also been documented [66] which has a low homology with the eukaryotic equivalent, therefore there is some uncertainty whether or not cyanobacteria contain the PsbW protein. In higher plants and green algae, it is nuclear-encoded and is processed to a mature protein having an apparent molecular mass of 6.1 kDa [61,67] and consists of 54–56 amino acids [68]. It is predicted to have one membrane-spanning region with its N-terminus located on the lumenal side of the membrane. This orientation is consistent with the pre-sequence of the PsbW protein having characteristics of a target sequence typical for lumenal proteins [69]. The function of PsbW is unknown although, interestingly, it is degraded under the same illumination conditions that degrade the D1 protein [70]. Moreover the disruption of the *psb*W gene of *Arabidopsis* is reported to disturb dimerisation of the PSII core dimer [71].

4.14. PsbX

The PsbX protein is nuclear-encoded in higher plants and green algae [57,61] and consists of about 42 amino acids. In higher plants its synthesis is tightly regulated by light [72]. The mature PsbX protein is reported to have a molecular mass of 4.1 kDa, and predicted to have a single transmembrane helix [73]. It has been detected in the oxygen-evolving PSII core complex of higher plants and cyanobacteria [55,59] but is not contained in isolated RC preparations [72]. The deletion of the *psb*X gene in the cyanobacteria *Synechocystis* [74] and *S. elongatus* [75] did not inhibit PSII assembly and photoautotrophic growth. However, these studies suggest that PsbX may play a role in the function of Q_A and Q_B.

4.15. PsbY

It has been proposed that the product of the *psb*Y nuclear gene (also known as L-AME) belongs to PSII [76]. The gene encodes two proteins, PsbY-A1 and PsbY-A2, which are separated after translation [77]. They were claimed to be Mn binding polypeptides [76] however, deletion of the *psb*Y gene in *Synechocystis* did not impair the water oxidation process [78]. It therefore remains to be proven that the PsbY proteins are subunits of PSII.

5. Localisation of the LMW subunits of the PSII core dimer

The structures of PSII determined for spinach [1,2] and *S. elongatus* [3] are both of insufficient resolution to allow detailed side chain information to be obtained and therefore prevent the direct assignment of LMW proteins to transmembrane densities. However, some biochemical evidence is available to aid the localisation of these LMW polypeptides within the PSII core dimer.

Based on the cyanobacterial structure [3] we have revised the assignment of the LMW proteins of the CP47–RC subcomplex from seven to five (see dark blue section of Fig. 4A). We have assumed that the densities immediately adjacent to helix B of the D1 and D2 proteins are not transmembrane helices as previously thought [1] but are due to the presence of chlorophylls ligated to D1His118 and D2His118. The revised number of LMW subunits in the CP47–RC subcomplex is consistent with mass spectrometry data and N-terminal sequencing analyses of an isolated and monomeric form of this CP47–RC subcomplex, which identified the five LMW proteins: PsbE, PsbF, PsbI, $PsbT_c$ and PsbW [64]. Consequently, these five proteins are assigned to the two purple and three grey helices in the CP47–RC domain of the core dimer (dark blue domain Fig. 4A).

Although of limited resolution, the 3D structure of PSII from *S. elongatus* did unambiguously identify the transmembrane helices of the PsbE and PsbF components of cyt b_{559}, by locating the haem Fe^{2+} and resolving the differences in their lumenally exposed C-terminal extensions. Based on their location in the cyanobacterial structure [3] and on previous studies [21] the two purple helices in Fig. 4A are assigned to

PsbE and PsbF as shown. With two of the five LMW protein helices of the CP47–RC subcomplex accounted for, the three remaining LMW proteins, PsbI, $PsbT_c$ and PsbW, can be attributed to the grey helices in the dark blue domain of Fig. 4A. The PsbI protein has been shown to cross-link both with the D2 protein and cyt b_{559}, suggesting it to be on the D2 rather than the D1 side of the RC [49]. Furthermore, the PsbI protein is tightly associated with the isolated D1/D2 heterodimer while PsbW and $PsbT_c$ are less so [48,79]. Consequently we tentatively assigned PsbI to the grey helix, closest to the D2 protein and cyt b_{559}. Based on this assignment and on the report that PsbW can also be cross-linked to the α-subunit of cyt b_{559} (PsbE) [80] as well as co-purifying with the D1/D2/cyt b_{559} RC complex [79], the second grey helix on the D2 side has been tentatively designated PsbW. By elimination, $PsbT_c$ can therefore be assigned to the last grey helix in the CP47–RC subcomplex located adjacent to helices A and B of the D1 protein (see Fig. 4A).

In addition to the five LMW proteins within the CP47–RC subcomplex, seven other LMW proteins have been attributed to membrane-spanning densities identified in the higher plant PSII core dimer (see Fig. 2). These additional LMW proteins are indicated as grey rods within the cyan domain of Fig. 4A. Of these seven proteins, four have been identified in our spinach dimer core complex by N-terminal sequencing and mass spectrometry: PsbH, PsbL, PsbK and PsbX. PsbL and PsbK were also previously found in dimeric, but not monomeric, forms of the spinach CP47–RC complex [64]. This suggests that PsbL and PsbK are likely to be located in the central cyan regions linking the two CP47–RC subcomplexes (Fig. 4A). They may therefore account for two of the three transmembrane helices in this central linker region. In this context it is interesting to note that the pseudo-two-fold symmetry axis which relates the D1 and D2 proteins, as well as CP47 and CP43, also approximately relates PsbE and PsbF with two of the grey helices in this central domain found adjacent to helix A of the D1 protein. Furthermore, in most organisms the PsbE, PsbF, PsbJ and PsbL are all encoded by a single chloroplast operon and expressed on a tetracistronic message opening up the intriguing possibility that the helix pair that is approximately symmetry-related to PsbE and PsbF consists of PsbJ and PsbL. Also as noted in Table 1, PsbJ and PsbL have highly conserved amino acid sequences. Consequently these two helices have been labelled J/L placing PsbK closest to the contact point of the PSII core dimer (Fig. 4A).

The two remaining LMW proteins that have been identified in the higher plant PSII core dimer are PsbH and PsbX. Recently the PsbH protein was shown to cross-link with the PsbE subunit of cyt b_{559} in PSII core dimers isolated from the green alga *Chlamydomonas reinhardtii* [80]. Using a combination of gold labelling and single particle analysis, it was confirmed that the PsbH subunit was localised to a position close to cyt b_{559}. As the PsbX subunit has been shown to be cross-linked with PsbH [80], as well as with PsbE [80,81], it is suggested that the two helices close to PsbE and PsbF are either PsbX or PsbH. This leaves two unassigned transmembrane helices in the cyan regions of the PSII core dimer. The first of these is adjacent to the helix that we have assigned to $PsbT_c$ with the second located close to helices 1 and 2 of CP43. The remaining two gene products that have been suggested to be part of the hydrophobic core of the PSII complex are PsbM and PsbN. Therefore we tentatively assign these helices to M and N as shown in Fig. 4A.

6. Comparing the PSII structures of higher plants and cyanobacteria

Fig. 4 compares the structure of higher plant PSII (spinach), derived from electron crystallography [2], with that of cyanobacterial PSII (*S. elongatus*) derived from X-ray crystallography [3] at the level of transmembrane helix organisation. Both structures show PSII to be dimeric with essentially identical interfaces between the two monomers. Also the position of the major subunits, D1, D2, CP43, CP47 and cyt b_{559}, and their corresponding transmembrane helices are the same. The three LMW proteins at the interface of the monomer and within the dimer are conserved between the two organisms. We assign these to PsbJ, PsbK and PsbL whereas Zouni et al. [3] tentatively assigned these to PsbH, PsbK and PsbL (Fig. 4B). The transmembrane helices near cyt b_{559} seem to be present in both structures. Although the assignment of PsbI is the same in both models, the helix assigned to PsbX in cyanobacteria could be PsbH, based on gold labelling and cross-linking experiments [80]. Both structures have a transmembrane helix adjacent to the B helix of the D1 and D2 proteins, which were unassigned by Zouni et al. [3], but we here assign to $PsbT_c$ and PsbW respectively. The fact that we assign PsbW to a helix which is also present in the structure of *S. elongatus* raises the possibility that cyanobacteria do contain this protein or an equivalent. The similarities and differences between the two structures are highlighted in Fig. 4B by colouring the common regions in the spinach and *S. elongatus* dimers in cyan. It can be seen that in higher plants the system has an additional helix adjacent to the B helix of the D1 protein (highlighted in Fig. 4A). In contrast, the cyanobacterial structure contains four transmembrane helices close to helices 1 and 2 of CP43 while only one helix was identified in the spinach structure (labelled M/N in Fig. 4A). The absence of these three additional cyanobacterial helices in the higher plant structure was confirmed by a closer inspection of the packing of dimeric complexes of spinach within the 2D crystals where it was found that there was no available space within the crystal lattice to account for these additional helices. We therefore conclude that although the two structures are extremely similar there are differences between the higher plant and cyanobacterial systems in terms of LMW protein content and location. These differences may relate to their different light harvesting systems [3] and lumenal extrinsic proteins or possibly reflect the thermostability of *S. elongatus*.

7. Final comment

It is particularly pleasing that electron crystallographic analyses have provided structural information of PSII that is very consistent with that derived by X-ray crystallography. Moreover, this consistency has shown that PSII RC cores of higher plants and cyanobacteria are structurally very similar at the level of both their oligomeric states (dimers) and the organisation of the transmembrane helices of their major subunits. In the case of the LMW subunits there seem to be some differences. The challenge of the future is to obtain higher resolution structures of plant and cyanobacterial PSII such

that amino acid side chains can be traced. In this way the nature of the protein environment surrounding the light harvesting and redox active cofactors will be revealed. This will be particularly important for the unique features of PSII such as the high potential chl *a* molecules which constitute P680 and the $(Mn)_4$ cluster which catalyses the water oxidation process. Also at this level of structural resolution, the position of LMW proteins will be unambiguously resolved and the nature of differences between higher plant and cyanobacterial PSII will be revealed. This high resolution information will probably come from X-ray diffraction analyses rather than from electron crystallography. However, there remains the challenge of understanding how the light harvesting antenna systems (e.g. phycobilisomes, LHCII) structurally and functionally couple to the RC cores to form supercomplexes. Here electron microscopy can contribute either by analyses of 2D crystals or by single particle analyses. Moreover, PSII is highly dynamic particularly in terms of the rapid turnover of the D1 protein due to photoinduced damage [14]. Electron microscopy, particularly single particle analyses, could contribute greatly to the understanding of structural changes that must occur as PSII goes through the damage repair cycle. Clearly then, structural studies of PSII will continue to benefit from a combination of X-ray crystallography and high resolution electron microscopy.

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