Refinement of the structural model for the Photosystem II supercomplex of higher plants

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Abstract

Recent X-ray structures determined for the Photosystem II (PSII) core complex isolated from cyanobacteria have provided important information for understanding the functionality of this photosynthetic enzyme including its water splitting activity. As yet, no high-resolution structure is available for PSII of plants or eukaryotes in general. However, crystal structures have been determined for some components of plant PSII which together with the cyanobacterial structure can be used to interpret lower resolution structures of plant PSII derived from electron cryomicroscopy (cryo-EM). Here, we utilise the published X-ray structures of a cyanobacterial PSII core, Light Harvesting Complex II (LHCII), PsbP and PsbQ proteins to construct a model of the plant LHCII–PSII supercomplex using a 17 Å resolution 3D electron density map of the spinach supercomplex determined by cryo-EM and single particle analysis. In so doing, we tentatively identify the relative positioning of the chlorophylls within the supercomplex and consider energy transfer pathways between the different subunits. The modelling has also allowed density to be assigned to the three extrinsic proteins of plant PSII, PsbO, PsbP and PsbQ associated with the water splitting centre and concluded that although the position of PsbO is the same as in cyanobacteria, PsbP and PsbQ are located in different positions to the cyanobacterial extrinsic PsbU and PsbV proteins.

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1. Introduction

About 2.5 billion years ago, photosynthetic organisms developed the machinery to oxidise water into dioxygen and reducing equivalents [1]. This heralded the ‘big bang’ of evolution because life on our planet was no longer limited by the supply of hydrogen donor substrates such as organic acids, Fe^{2+}, H_{2}S and NH_{2}. Water was, and still is, available in essentially unlimited amounts. Nature had found the perfect solution of using solar energy to split water and thus provide the carbon fixation process of photosynthesis with an endless supply of reducing equivalents. It was this solution that is responsible for the enormous amount of biomass on our planet today and is, of course, the origin of our fossil fuels. The release of dioxygen created an oxygenic atmosphere allowing respiration to maximise its efficiency.

It is not yet understood how the photosynthetic water splitting enzyme evolved. The reaction takes place in a membrane-located multisubunit complex known as Photosystem II (PSII) [2]. Recent X-ray structures of cyanobacterial PSII have been obtained [3–6] at sufficient resolution to assign side chains and model the catalytic centre where the water splitting reaction occurs [5,6]. However, no such high-resolution information is available for PSII of eukaryotes although poorly diffracting 3D crystals of the isolated complex have been reported [7,8]. The highest resolution structure of the plant PSII core complex has been obtained by electron crystallography which led to the assignment of the positioning of the major subunits and location of transmembrane helices [9–12]. Overall, at the intermediate resolution attained (8 to 9 Å), there is good agreement between the models derived for higher plants and cyanobacteria except for the number of transmembrane helices attributed to low molecular subunits of PSII [13].

Lower resolution 3D structures of PSII from higher plants, cyanobacteria and green algae have been obtained by single
particle analyses [14–16]. In particular, a 3D structure of higher plant supercomplex consisting of PSII dimeric core and light harvesting complex (LHCII) was obtained from electron cryo-microscopy (cryo-EM) [16]. In addition to binding two trimers of LHCII, this isolated supercomplex contains two copies of the minor Cab proteins CP29 and CP26, where each pair is symmetrically related by the 2-fold axis of the dimer [17,18].

It was estimated that this supercomplex bound a total of approximately 100 chlorophylls (Chl) per PSII reaction centre, about 75 being Chl a and the remaining Chl b [19], with the total molecular weight of the dimeric LHCII–PSII supercomplex being in the region of 1100 kDa. The electron density map of the LHCII–PSII supercomplex has provided a framework for incorporation of high-resolution structural information derived from electron crystallography. In this way, assignments were made for the positioning of major subunits of PSII, for the LHCII trimer and for the minor Cab proteins including their transmembrane helices [17,18]. These assignments have provided a model for discussing the overall organisation of LHCII–PSII supercomplexes and other LHCII and Cab (CP24) proteins in the intact thylakoid membrane [20]. An improved 3D structure of the LHCII–PSII supercomplex, also derived from single particle analysis [21], revealed further density for the extrinsic proteins of the oxygen evolving complex (OEC). Interpretation of this density was, however, more difficult since high-resolution structures were not available for these OEC extrinsic proteins of PSII of higher plants at that time.

Here, we refine the structural model of the plant LHCII–PSII supercomplex using high-resolution X-ray structures of the PSII core [5], LHCII [22] and extrinsic OEC proteins [5,22–24] through use of the PyMol software environment (DeLano Scientific, http://www.pymol.org). In so doing we explore relationships between the pigments within the supercomplex and also the organisation of its OEC extrinsic proteins.

2. Core complex

The PSII dimeric core complex of higher plants, algae and cyanobacteria seem to be structurally very similar [12,13]. At the heart of each monomer are the D1 and D2 reaction centre proteins, which bind the cofactors that facilitate the light driven charge separation leading to water oxidation and the reduction of plastoquinone [2]. Each has five transmembrane helices related to each other by a pseudo-2-fold axis. Flanking each side of the D1 and D2 proteins are the Chl-binding proteins CP43 and CP47. These have six transmembrane helices each, which are related also to each other by the same pseudo-2-fold axis of the D1/D2 heterodimer. Surrounding these symmetrically related major subunits are a number of low molecular weight transmembrane proteins. Attached to the luminal surface of PSII are the OEC proteins. The structural relationships of these subunits are shown in Fig. 1 based on X-ray crystallography of the PSII core isolated from the cyanobacterium, *Thermosynechococcus elongatus* [5]. The model obtained was sufficiently refined to trace the majority of the side chains of the various protein subunits and therefore provided the first description of the protein environment of the various cofactors involved in...
PSII function, including those that catalyse the water splitting reaction. The latter was modelled as a Mn$_3$Ca$^2+$O$_4$ cubane with a fourth Mn ion linked to the cubane via a $\mu$-oxo bridge (see Fig. 2b). Successive oxidations of the metal centre are driven by charge separation between the primary electron donor P680 (composed of the PD1 and PD2 Chls and possibly Chl$_{D1}$ and Chl$_{D2}$) [25,26] and the terminal plastoquinone acceptor Q$_B$ (Fig. 2a). The oxidation reactions are mediated by a redox active tyrosine of the D1 protein (Tyr$_2$) while the reductive pathway involves a pheophytin molecule (Pheo$_{D1}$) and a firmly bound plastoquinone Q$_A$. Pheo$_{D2}$ is not involved in charge separation while the other cofactors shown in Fig. 2a play a role in the protection and regulation of the reaction centre [27,28]. Cyt b559 is a high potential haem ligated via histidines to two low molecular weight proteins, PsbE and PsbF, often known as the $\alpha$- and $\beta$-subunits. All the other cofactors are associated with the D1 and D2 proteins, except one of the Mn ions of the Mn$_3$Ca$^2+$O$_4$ cubane has a ligand provided by CP43 (see Fig. 2b).

Ferreira et al. [5] assigned 16 Chls and 14 Chls bound to CP47 and CP43, respectively, which together with the 6 Chls associated with the D1/D2 heterodimer gives a total of 36 Chls for each monomer within the dimeric core complex (see Fig. 1c). These Chls provide a light harvesting system for the reaction centre and are arranged in two layers towards the stromal and luminal surfaces of the complex except for one Chl in each subunit, which bridges between the two layers. Both proteins are distinguished in having large lumenal loops joining transmembrane helices V and VI. In the case of CP43 a 310 helix contained within this extrinsic domain provides the ligand for Mn3 of the metal cluster (CP43 Glu354) (see Fig. 2b) and an arginine (CP43 Arg357) which is strategically positioned in the catalytic cavity.

Other than the $\alpha$- and $\beta$-subunits of Cyt b559, none of the low molecular weight intrinsic proteins directly bind Chls or redox active cofactors. They either form a dimerisation domain (PsbL, PsbM, PsbF), stabilise the binding of Chls (Chl$_{ZD1}$ by PsbI and Chl$_{ZD2}$ by PsbX) or carotenoids (PsbJ, PsbK, PsbN and PsbK) (assignment of PsbN is tentative and could possibly be PsbY) [5]. In cyanobacteria, the extrinsic OEC proteins that form a stabilising ‘cap’ over the Mn$_4$Ca$^2+$O$_4$ cluster are PsbO, PsbU and PsbV and their location in side elevation can be seen in Fig. 1a.

A recent 3.0 Å crystal structure of cyanobacterial PSII [6] is consistent with that of [5] and provides additional information about lipid and carotenoid locations. Differences include the presence of an extra low molecular weight transmembrane subunit and the assignment of density to a lipid molecule rather than a Chl in CP43 giving a total of 35, rather than 36, Chls per monomeric PSII core.

In the absence of a high-resolution structure of the plant PSII core, we have chosen to use the cyanobacterial X-ray structure for modelling the core into the electron density of the 17 Å resolved spinach LHCCI–PSII supercomplex [21]. As a consequence we are able to position the Chls of the dimeric core within the plant LHCCI–PSII supercomplex and assign density to the PsbO protein with some degree of confidence.

3. Light harvesting systems

3.1. Chlorophyll organisation

There have been two high-resolution crystal structures determined for LHCCI isolated either from spinach [22] or pea...
Previous modelling of LHCII, CP29 and CP26 into the spinach LHCII–PSII supercomplex was based on the intermediate resolution model determined by electron crystallography [30]. Using the new data for LHCII and the X-ray structure of the core complex, we can get a better model for the organisation of the Chls within the LHCII–PSII supercomplex and possibly open up discussions about energy transfer pathways. Fig. 3 shows an overlay of the X-ray structures of spinach LHCII [22] and the cyanobacterial PSII core [5] onto the 17 Å projection map of the cryo-EM 3D structure of the isolated LHCII–PSII supercomplex of spinach [21]. The positioning and orientations of the X-ray structures within the cryo-EM map are consistent with previous modelling of the electron crystallographic structures of LHCII and PSII core [21] and refined using features in the electron density assigned to these components in a 2D projection map derived from electron crystallography (Barber and Morris unpublished). Similarly, the approximate positioning of CP29 and CP26 are the same as before [16,21] except in Fig. 3, the X-ray structure of a monomeric LHCII protein was used to model the backbone of these Cab proteins by extracting the relevant atoms from the 1RWT.pdb coordinates [22]. The fitting of these minor Cab proteins took into account the electron density features attributed to them in the cryo-EM 3D map. Also recognised are reports that CP26 and CP29 contain less Chls and have a different Chla/Chlb ratio compared with LHCII. Croce et al. [31] concluded that CP26 binds 9 Chls (6 Chla, 3 Chlb), contrasting with a monomer of LHCII which binds 14 Chls (8 Chla, 6 Chlb). Based on the numbering of Liu et al. [22], the 6 Chl of CP26 correspond to the LHCII Chls 602, 603, 610, 612, 613 and 614 while the Chlb of CP26 are equivalent to 609, 606 and 611. It seems that CP29 has the same Chla/Chlb binding sites as CP26 except that it probably does not have the 611 Chlb and therefore binds overall one less Chlb than CP26 [31,32].

Although the cryo-EM map is at 17 Å resolution compared to X-ray structures solved from 2 to 4 Å, suitable margins for error should be considered, but the overall fitting, i.e., as shown in Fig. 3, is consistent to at least ± 1 nm and thus the model gives an opportunity to discuss within the limitations of the resolution how the chlorophylls of LHCII, CP29 and CP26 spatially relate with each other and with those bound to CP47 and CP43, at either end of each PSII monomer. Fig. 4 thus shows the arrangements of the chlorophylls within the subunits of the full supercomplex represented as modelled atomic structures, coloured grey in Fig. 4a.

Given that each LHCII monomer binds 14 Chls of which 8 are Chla and 6 are Chlb [22,29] and that CP29 binds 8 Chls (6 Chla, 2 Chlb) and CP26 binds 9 Chls (6 Chla, 3 Chlb) [31,32], then together with the 36 Chls per monomer of PSII [5] the complete supercomplex binds a total of 190 Chls or 95 Chls per PSII reaction centre where the Chla/Chlb ratio is 3.13. This agrees with the earlier biochemical analyses [19] as does the estimate of 72 Chla and 23 Chlb molecules per PSII reaction centre.

The overall characteristic of the Chl organisation in the supercomplex is that they are distributed mainly in two layers (Fig. 4c), one being on the luminal side and the other towards the stromal surface. However, according to the modelling the distances between the Chls of the peripheral Cab antenna complexes and those of CP43 and CP47 are rather long being in most cases greater than 20 Å (Fig. 4b). It has been proposed that the Chla cluster Chl610, Chl611 and Chl612 of LHCII are excitonically coupled and that they are the terminal site for energy transfer within LHCII [22,33,34] and therefore likely to be directly involved in energy transfer to the PSII core. Indeed, the model indicates that LHCII Chl 612 is about 17 Å from Chl 11 of CP43 (ringed in Fig. 4b). Chl 612 has been suggested to be the terminal fluorescence emitter of LHCII [22] which would be
consistent with its suggested role in facilitating energy transfer to CP43 although Chl 610 could also function this way according to recent calculations [34]. If this is the case, then presumably the other two clusters of Chl 610–Chl 612 of the LHCII trimer are involved in aiding energy transfer from adjacent LHCII trimers which would be attached to the LHCII–PSII supercomplex in the intact thylakoid membrane [20]. Despite the relative long distances between the Chla molecules of the peripheral antenna and those of the PSII core, the model shows that Chlb 605 of LHCII is positioned between CP26 and CP29. In fact in the latter case Chlb 605 it is closest to CP29 Chla 614 than it is to Chla of LHCII (Chl 604) by almost 5 Å edge-to-edge. Clearly it is unlikely to facilitate energy transfer from LHCII to CP29 or to CP26, although Pascal and colleagues [35] recently highlighted its close interaction with Chl 614 in the adjacent LHCII complex within the icosahedral proteoliposomes of the 3D crystals used to obtain the atomic structure [22].

The rather long distances between the Chla molecules of the peripheral Cab proteins and those of PSII core raises the possibility that there are “linker” Chls bridging the gaps as found for the LHCI–PSI supercomplex [36]. In this context, an electron crystallographic analysis of the dimeric PSII core complex isolated from spinach indicated the presence of an additional single transmembrane helix adjacent to the PsbI subunit which is not present in cyanobacterial PSII [12]. It was suggested that this protein may bind Chl and in this way facilitate energy transfer from LHCII [12].

This possibility and the whether other linker Chls are present in the LHCII–PSII supercomplex of plants and green algae can only be revealed by better structural resolution than available at present.
4. Extrinsic proteins

4.1. PsbO

This protein is present in all known oxygenic photosynthetic organisms [37] and its structure seems to be conserved between the eukaryotic and prokaryotic forms except for some minor differences [38]. It consists of a \( \beta \)-barrel made up of eight anti-parallel \( \beta \)-strands with a large loop joining strands 5 and 6 that forms a large ‘head’ domain. This domain contains several highly conserved motifs which are involved in binding PsbO to the lumenal surface of PSII. In this way the PsbO protein stabilises the peptides that are close to the catalytic water splitting-oxygen evolving centre [5,38]. It is clear that the incorporation of the cyanobacterial X-ray structure into the electron density map of the spinach supercomplex, derived from cryo-EM, places the PsbO protein (white) in the protruding ‘ear-like’ feature on the lumenal surface (Fig. 5c). Moreover, this modelling indicates that the large extrinsic loop of CP47 (red) is located in the density which bridges between the two main bodies assigned to PsbO protein (Figs. 5b and 6) of the dimeric complex while density due to the large extrinsic loop of CP43 (green) is to one side of the PsbO protein.

4.2. Other OEC proteins

Although cyanobacteria and plants contain the PsbO protein the other extrinsic proteins are different. Cyanobacteria contain the PsbU and PsbV, where the latter is a cytochrome (Cyt c550), while plants contain the PsbP and PsbQ proteins [37,39]. However, under some circumstances cyanobacterial PSII may bind a PsbQ-like protein and possibly a PsbP-like protein [40]. When the cyanobacterial X-ray structure is built into the electron density of the spinach LHCII–PSII supercomplex, there is no corresponding density for PsbU or PsbV as clearly seen in Fig. 5c, d. We therefore assume that the remaining extrinsic density in the EM map of the LHCII–PSII supercomplex is due to PsbP and PsbQ. X-ray structures of the isolated PsbP, 1V2B.pdb [24] and PsbQ, 1NZE.pdb [23] proteins have recently been reported. The PsbP protein was isolated from *Nicotiana tabacum* and shown to be mainly composed of a \( \beta \)-sheet. In contrast the core of the PsbQ protein, isolated from spinach, is a four-helix bundle. Assuming that these structures of the isolated proteins are approximately the same as in their bound state, they can be used to assign the remaining lumenal density of the LHCII–PSII supercomplex.

Experimental evidence suggests that the PsbP cannot bind efficiently in the absence of PsbO. Similarly PsbQ requires PsbP to be present [38]. We have taken this into account when modelling the X-ray structures of these two extrinsic proteins into the electron density of the LHCII–PSII supercomplex (see Figs. 5 and 6). Although there is density in the cryo-EM map to accommodate most of the structure of these two proteins within the lumenal “ear-like” feature which contain PsbO, there is some short fall which reflects

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Fig. 5. Views of the LHCII–PSII supercomplex to emphasise the fitting of X-ray structures of the LHCII and OEC extrinsic proteins into the 3D electron density map of the spinach supercomplex (a) Lumenal top view with X-ray structure of the cyanobacterial PSII core complex [5] emphasising the positioning of PsbO (white spheres), PsbU (cyan spheres), PsbV (blue spheres), PsbP (magenta stick representation), PsbQ (yellow stick representation). (b) Side view of (a) but showing electron density envelope of the supercomplex to emphasise the extrinsic density on the lumenal surface. Colours as in (a), (c) surface rendered lumenal top view emphasising that most of the density attributed to the structure of PsbO [5], PsbP [24] and PsbQ [23] is contained within the molecular envelope of the supercomplex and that there is no density to accommodate or replace the PsbU (cyan) or PsbV (blue). (d) Surface rendered side view corresponding to (b) supporting the conclusions of (c) concerning PsbU and PsbV. Note that the comparison of (b) and (d) shows that the density between the two PsbO proteins contains the large extrinsic loop of CP47, coloured in red.
the quality of the low resolution cryo-EM map, possibly because of disorder or reduced occupancy in some of the LHCII–PSII supercomplexes used in single particle averaging. Bearing in mind the uncertainties of the assignments, the modelling suggests that the PsbP protein not only interacts with the PsbO but also with the lumenal surface of the PSII core in the vicinity of CP43. Indeed, there is some evidence that PsbP can bind weakly to the PSII surface in the absence of PsbO [41]. In the case of PsbQ our modelling suggests that it bridges between the PsbP and the PsbO proteins (see Fig. 6).

5. Discussion

By taking advantage of the most recent structural information we have attempted to provide a working model for the structure of the plant LHCII–PSII supercomplex. Based on X-ray structural information for the cyanobacterial PSII core [5,6] and for plant LHCII [22] as well as detailed analyses of CP26 and CP29 [31,32], we can conclude that this supercomplex binds at least 95 Chls (72 Chla and 23 Chlb molecules) per reaction centre and somewhere in the region of 30 carotenoids. We had hoped to identified specific Chls which could be important in enabling energy transfer from the outer light harvesting system composed of a LHCII trimer, CP29 and CP26, to the PSII reaction centre via the Chls of CP43 and CP47. Our modelling is consistent with the proposal that the LHCII Chla-cluster (Chl 610–612) is involved in transferring excitation energy to the PSII reaction centre via CP43. However, no obvious energy transfer routes were identified between LHCII and CP26 and CP29 or between CP26 and CP29 and the PSII core, since the closest Chla–Chla intersubunit distances were 20 Å or more. It also seems unlikely that the LHCII Chlb (Chl 605) positioned between LHCII and CP29 or CP26 could play this role. This raises the possibility that linker Chls may exist to bridge the gap as found in the case of the LHCI–PSI supercomplex [36].

Although the intrinsic components, both proteins and cofactors, are likely to be structurally very similar in the PSII cores of cyanobacteria and plants, there is a striking difference in the nature of their OEC extrinsic proteins. The PsbO protein is present in all cases and our analysis suggests that its position and structure in plant PSII is essentially as it is in cyanobacterial PSII. The location of the plant PsbP and PsbQ proteins, however, seems to be very different to that of PsbU and PsbV found in the PSII of cyanobacteria. There is no electron density to accommodate the PsbU and PsbV proteins and, indeed, there is no evidence that these proteins exist in plants. Our results therefore do not support the concept that the PsbP and PsbQ are structural and functional replacements for the cyanobacterial PsbV and PsbU [40].

As yet, there is no structural information available for a cyanobacterial PSII core complex binding all five extrinsic OEC proteins but our work suggests that this complex could...
exist assuming the cyanobacterial PsbP-like and PsbQ-like proteins associate with PSII in the same way as their higher plant counterparts.

A side view of our structural model for the spinach LHCCI–PIT supercomplex is shown in Fig. 7 and emphasises the suggested arrangement of the three extrinsic OEC proteins. Within the limitations of the resolution of the cryo-EM map for the supercomplex we can be confident that the positioning of the PsbO is reasonably accurate. The density which accommodates its β-barrel is clearly seen as a protruding ear-like feature on the luminal surface of the supercomplex. It seems that this density is also sufficient to accommodate most of the PsbP and PsbQ proteins and also some of the large extrinsic loop of CP43. As can be seen in Figs. 6 and 7, PsbP is modelled towards the luminal surface of PSII and seems to interact with loops of CP43 and possibly with the C-terminus of the D1 protein. On the other hand, PsbQ forms a bridge across from PsbO and PsbP. In this way, PsbQ is rather like PsbU which bridges between PsbO and PsbV in cyanobacteria [4–6].

Clearly, the precise positioning of PsbP and PsbQ within plant PSII will only emerge from high-resolution X-ray structural analyses but the organisation shown in Fig. 7 provides a basis to continue to explore the role of these extrinsic proteins in PSII function and dynamics. For example, there are recent reports that plant PsbO binds GTP and could function as a GTPase [42] and that PsbP has structural characteristics which suggest that it might be a GTPase activating protein [24]. Moreover, plant PsbO has been suggested to act as a carbonic anhydrase [43]. In time a better understanding of the functional role of these plant OEC extrinsic proteins will emerge. Finally the X-ray structure of the cyanobacterial PSII core revealed a hydrophilic channel leading from the OEC to the luminal surface [5]. This channel passes across the ‘neck’ region of the PsbO (between the β-barrel body and the extended loop domain joining β-strands 5 and 6, see [38]. The exit point is at PsbO Gln229 (T. elongatus sequence) and is not impeded by PsbU or PsbV. Similarly our modelling of plant PSII also suggests that the exit to this channel is not blocked by either PsbP or PsbQ.

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