Review

Photosynthetic generation of oxygen

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The oxygen in the atmosphere is derived from light-driven oxidation of water at a catalytic centre contained within a multi-subunit enzyme known as photosystem II (PSII). PSII is located in the photosynthetic membranes of plants, algae and cyanobacteria and its oxygen-evolving centre (OEC) consists of four manganese ions and a calcium ion surrounded by a highly conserved protein environment. Recently, the structure of PSII was elucidated by X-ray crystallography thus revealing details of the molecular architecture of the OEC. This structural information, coupled with an extensive knowledge base derived from a wide range of biophysical, biochemical and molecular biological studies, has provided a framework for understanding the chemistry of photosynthetic oxygen generation as well as opening up debate about its evolutionary origin.

Keywords: oxygen-evolving centre; water oxidation; structure; photosystem II; manganese cluster; evolution

1. PHOTOSYSTEM II (PSII): WHERE ATMOSPHERIC OXYGEN IS GENERATED

Somewhere in the region of 2.5 Ga ago, an enzyme emerged which would dramatically change the chemical composition of our atmosphere and set in motion an unprecedented explosion in biological activity. This enzyme used solar energy to power the thermodynamically and chemically demanding reaction of water splitting. In so doing, it provided biology with an unlimited supply of ‘hydrogen’ (in the form of reducing equivalents) needed to convert carbon dioxide, initially into sugars (CH₂O) and then into the other organic molecules of life.

Prior to this, biology had been dependent on hydrogen/electron donors, such as H₂S, NH₃, organic acids and Fe²⁺, which were in limited supply compared with the ‘oceans’ of water with which planet Earth is blessed. The by-product of the water-splitting reaction is molecular oxygen. The release of this gas also had dramatic consequences for biology since it converted our planet from being anaerobic to aerobic and led ultimately to the formation of the ozone layer. With oxygen available, the efficiency of metabolism increased dramatically since aerobic respiration provides in the region of 20 times more cellular energy than anaerobic respiration. It was probably this improved efficiency due to aerobic metabolism which drove the subsequent evolution of eukaryotic cells and multicellular organisms. The establishment of the

ozone layer provided a shield against harmful UV radiation allowing organisms to explore new habitats and especially to exploit the terrestrial environment. In a nutshell, when biology learnt to split water using sunlight it had solved its energy problem allowing life to prosper and diversify on an enormous scale as witnessed by the fossil records and by the extent and variety of living organisms on our planet today. The enzyme that gave rise to this ‘big bang of evolution’ is known as photosystem II (PSII) and is therefore an enzyme of global and evolutionary significance. It is a multiprotein complex contained within the thylakoid membranes of all types of plants, algae and cyanobacteria (Diner & Babcock 1996; Barber 2003; Wydrzynski & Satoh 2005). In contrast to chemical and electrochemical water splitting which are thermodynamically highly demanding, the PSII-catalysed biological water-splitting mechanism is truly remarkable since it proceeds with very little driving force and requires only moderate activation energies.

2. THE REACTIONS OF PSII

Over the years, a wide range of biochemical and biophysical techniques have provided a good understanding of the events that power the oxidation of water and the resulting generation of molecular oxygen (reviewed in the various chapters of Wydrzynski & Satoh 2005). These processes are initiated through the absorption of light energy by many chlorophyll and other pigment molecules associated with PSII. The nature of these PSII light-harvesting systems varies under different growth conditions and with different types of organisms. However, within the PSII core complex, only chlorophyll *a* (Chl *a*) and β-carotene are found, bound mainly to CP43 and CP47 proteins. In total, there are approximately 36 Chl *a* and 11 β-carotene per PSII core based on biochemical

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rstb.2008.0047> or via <http://journals.royalsociety.org>.

One contribution of 15 to a Discussion Meeting Issue ‘Photosynthetic and atmospheric evolution’.

(Barbato *et al.* 1991) and structural analyses (Zouni *et al.* 2001; Kamiya & Shen 2003; Ferreira *et al.* 2004; Loll *et al.* 2005).¹ The excitation energy absorbed by these pigments is 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. A special form of Chl *a* P functions as an exciton trap and is converted to a strong reducing agent after excitation (P^*). P^* reduces a pheophytin molecule (Pheo) within a few picoseconds to form the radical pair state $P^{\cdot+}Pheo^{\cdot-}$. Within a few hundred picoseconds, $Pheo^{\cdot-}$ reduces a firmly bound plastoquinone molecule (Q_A) to produce $P^{\cdot+}PheoQ_A^-$. $P^{\cdot+}$, which has a very high redox potential (>1 V), oxidizes a tyrosine residue (TyrZ) to form TyrZ $^{\cdot+}PheoQ_A^-$ on a nanosecond time scale. The oxidation of TyrZ is dependent on the deprotonation of its phenolic group to generate a neutral radical (TyrZ $^{\cdot}$). In the millisecond time, domain Q_A^- reduces a second plastoquinone (Q_B) to form Tyr $^{\cdot}PheoQ_AQ_B^-$. At about the same time, Tyr $^{\cdot}$ extracts an electron from a cluster of four manganese ions and a calcium ion (Mn_4Ca cluster) that bind two substrate water molecules. A second photochemical turnover reduces Q_B^- to $Q_B^{\cdot-}$, which is then protonated to plastoquinol and released from PSII into the lipid bilayer, subsequently oxidized by photosystem I (PSI) via the cytochrome b_6f complex. Two further photochemical turnovers provide four oxidizing equivalents required to oxidize two water molecules to generate dioxygen. Each oxidation state generated in the oxygen-evolving centre (OEC) is represented as an intermediate of the S-state cycle (Joliot *et al.* 1969; Kok *et al.* 1970) of which there are five distinct states (S0–S4). In addition to these reactions, side reactions can occur under some conditions including the oxidation of a high-potential cytochrome bound within the PSII core complex (Cyt b559), a β -carotene molecule and a Chl *a* molecule (Chl $_Z$; Stewart & Brudvig 1998; Faller *et al.* 2001; Telfer 2002; Tracewell & Brudvig 2003). These side reactions occur on the tens of millisecond time scale and therefore do not compete with the electron transfer pathway leading to water oxidation. Indeed, they probably only occur when the rate of water oxidation becomes limited and thus provide a protective mechanism against the detrimental reactions resulting from the very high redox potential of the long-lived P radical cation.

3. THE STRUCTURE OF PSII AND ITS OEC

A more detailed understanding of the reactions leading to dioxygen production has come from elucidating the structure of PSII. It had been shown by electron microscopy (EM) that the PSII RC core complex of plants and cyanobacteria is dimeric (reviewed in Hankamer *et al.* 1997). In fact, our early EM studies employing both electron crystallography (Rhee *et al.* 1997, 1998; Hankamer *et al.* 1999, 2001) and single particle analyses (Nield *et al.* 2000a,b, 2002) had also revealed the relative positions of the D1, D2, CP43 and CP47 proteins within each monomer of the dimeric PSII RC core complex isolated from higher plants

(spinach) and suggested how their transmembrane helices were arranged (Barber 2002). The best resolution obtained was 8 Å and thus densities could be tentatively assigned to Chls bound within the CP47 protein as well as those that were contained within the D1/D2 heterodimer (Rhee *et al.* 1998). For the same reason, it was possible to speculate which of the various single transmembrane densities also contained in the map could be assigned to those of PsbE and PsbF since the haem of Cyt b559 which they ligate is equivalent in size to that of the tetrapyrrole head group of Chl (approx. 7 Å in width). Although the EM studies provided the first glimpse of the structural organization of PSII, they did not give the high-resolution information necessary to describe the exact positioning of cofactors and the nature of their protein environments. This was provided later by several X-ray diffraction analyses yielding crystal structures at different degrees of completion (Zouni *et al.* 2001; Kamiya & Shen 2003; Ferreira *et al.* 2004; Loll *et al.* 2005). Using PSII isolated from the cyanobacterium *Thermosynechococcus elongatus*, Zouni *et al.* (2001) were able to grow three-dimensional crystals and obtain a structural model at a resolution of 3.8 Å. However, in this initial study, the tracing of C- α backbones of some subunits was not completed and amino acids were not assigned, other than a tentative identification of D1 Tyr161 (TyrZ) and D2 Tyr160 (Tyr $_D$). Despite this, Zouni *et al.* (2001) did provide information on the positioning of cofactors involved in excitation energy transfer and charge separation. Most importantly, the analysis of the diffraction data revealed the first direct hints of the position of the Mn_4 cluster within PSII, which was found to be towards the luminal surface of PSII on the D1 side of the pseudo-twofold axis relating the transmembrane helices of the D1 and D2 RC proteins and the cofactors they bind. In this way, Zouni *et al.* (2001) confirmed the expectation that the location of the Mn cluster breaks the pseudo-twofold symmetry of the PSII RC and therefore placed it in the vicinity of the redox active Tyr161 of the D1 protein (Yz) and close to the surface helix located in the loop joining the luminal ends of the C and D transmembrane helices of the D1 protein (CD helix). The electron density had a 'pear shape' and Mn ions were tentatively positioned in the three bulges of this density to form an isosceles triangle with a fourth Mn ion placed above the centre of the triangle. Another important outcome of this work was confirmation that Cyt b559 was located on the D2 side of the reaction centre.

The 3 + 1 organization of the four Mn ions modelled by Zouni *et al.* (2001) gave support to the arrangement previously suggested by Peloquin *et al.* (2000) and also earlier by Hasegawa *et al.* (1999) and was also a feature of the crystallographic model of the Mn cluster derived by Kamiya & Shen (2003). Using PSII isolated from the *Thermosynechococcus vulcanus*, a cyanobacterium closely related to *T. elongatus*, these workers obtained a crystal structure at 3.7 Å and provided additional information to that revealed by Zouni *et al.* (2001). The tracing of the main chains was more complete and there was some effort made to assign amino acids, particularly those of the D1 and D2 proteins, as well as to some regions of the chlorophyll-binding proteins,

Figure 1. The water-splitting site of PSII showing the $\text{Mn}_4\text{Ca}^{2+}$ cluster positioned within the Mn anomalous difference map of Ferreira *et al.* (2004). (a) Based on the model of Ferreira *et al.* (2004). (b) Schematic of the amino acid ligation pattern for model in (a) with distance less than 3 Å shown by connecting lines. (c) Remodelling the water-splitting site using the native electron density maps of Ferreira *et al.* (2004) and Loll *et al.* (2005) and Mn anomalous difference map of Ferreira *et al.* (2004), keeping the $\text{Mn}_3\text{Ca}^{2+}\text{O}_4$ cubane of Ferreira *et al.* but with Mn_4 linked to it via a single 3.3 Å mono- μ -oxo bridge (taken from Barber & Murray 2008). (d) Schematic of the amino acid ligation pattern for model in (c) with distance less than 3 Å shown by connecting lines. The Mn anomalous difference map is shown in red and contoured at 5 σ with the fitting of the metal ions into this density by real-space refinement using the molecular graphics programme, Coot (Emsley & Cowtan 2004). The arrow indicates the direction of the normal to the membrane plane.

CP43 and CP47. The positioning of cofactors was essentially the same as that reported by Zouni *et al.* (2001). Kamiya & Shen confirmed that electron density for the Mn cluster was 'pear-like' in shape and modelled the four Mn ions in approximately the same positions as Zouni *et al.* (2001) except for the central Mn ion. Importantly, Kamiya & Shen's map contained electron density connecting to that of the Mn cluster, which was tentatively assigned to side chains of the D1 protein including those which had been previously identified by site-directed mutagenesis (Debus 2001; Diner 2001). As in the case of the earlier crystal structure (Zouni *et al.* 2001), the model of Kamiya & Shen did not include a Ca^{2+} bound close to the Mn cluster.

The first complete and refined structure of PSII came from the work of Ferreira *et al.* (2004) where over 5000 amino acids were assigned in the dimeric complex. Consequently, this 3.5 Å model identified and detailed the structures of 19 different subunits of the *T. elongatus* PSII complex (except for one low-molecular weight intrinsic subunit tentatively assigned to PsbN, which is almost certainly PsbYcf12; Kashino *et al.* 2007; PsbYcf12 is now renamed Psb30). It also

provided the first reliable information about the protein environments of all the major cofactors of PSII, not only those of the OEC but also for those involved in energy capture, excitation transfer and charge separation. The 'pear-shaped' Mn anomalous difference map of Ferreira *et al.* (2004) correlated with one Mn atom in the small domain of this difference electron density map and three in the large globular domain of its pear shape, whereas the Ca^{2+} anomalous difference map suggested that Ca^{2+} was located adjacent to the large domain. In this way, three Mn ions and the Ca^{2+} were modelled as a trigonal pyramid with the Ca^{2+} at its apex, all located in the large domain. The fourth Mn ion was placed in the small domain (figure 1a).

The much improved quality of the electron density map of Ferreira *et al.* (2004) led to the assignment of virtually all the amino acids in the PSII complex and in particular those in close proximity to the Mn_4Ca cluster. The positioning of side chains, the analyses of the anomalous diffraction data and recognition that the ions were likely to be bridged by oxo bonds at distances suggested by extended X-ray absorption fine-edge spectroscopy (EXAFS) resulted in a detailed model for the Mn_4Ca cluster (figure 1). However, at the

resolution of the diffraction data, the precise positioning of the metal ions could not be determined. Nevertheless, calculations using quantum mechanics (Lundberg & Siegbahn 2004; Sproviero *et al.* 2006, 2007) and chemical synthesis of a mixed Mn/Ca complex (Misra *et al.* 2005) indicated that the model suggested by Ferreira *et al.* (2004) was chemically feasible despite there being no known similar structure in biology. Moreover, the model has provided an important basis for developing detailed mechanisms for the water-splitting reaction leading to dioxygen formation (McEvoy & Brudvig 2004, 2006; Siegbahn & Lundberg 2005; Siegbahn 2006).

Based on this organization of the metal ions, the Mn_3CaO_4 cubane had four protein side chains as ligands, namely D1Asp342 to Mn1, D1Glu189 and D1His332 for Mn2 and CP43Glu354 for Mn3 (figure 1). The identification of the glutamate of CP43 as an Mn ligand was a surprise and is a residue of a conserved motif Gly–Gly–Glu–Thr–Met–Arg–Phe–Trp–Asp, which forms a 3_{10} helix in the large extrinsic loop joining the luminal ends of trans-membrane helices V and V1 of this protein. In addition to these four apparent protein ligands, it was noted that the C-terminal residue of the D1 protein, D1Ala344 is located close to Ca^{2+} and that D1His337 could be hydrogen bonded to one of the bridging oxo bonds of the cubane. Two side chain densities were available as ligands for the ‘dangler’ Mn4 outside the cubane cluster. These were identified as D1Asp170 and D1Glu333 and also noted was that D1Asp61 might also function as a ligand via a bridging water molecule. Because the coordination number is usually six or seven for Ca^{2+} and five or six for Mn, then according to the Ferreira *et al.* model there would need to be additional non-protein ligands present, such as water molecules or hydroxides. Nevertheless, Ferreira *et al.* (2004) did emphasize that there was non-protein electron density in the vicinity of Mn4 and Ca^{2+} , which they tentatively assigned to a carbonate ion that formed bridging ligands between the two metals. Moreover, the positioning of this carbonate ion adjacent to the redox active D1Tyr161 (TyrZ) suggested that this could be the site for the binding of two substrate water molecules involved in the formation of dioxygen. Also located in this potential catalytic site are D1Gln165 and CP43Arg357, which may provide hydrogen-bonding networks for deprotonation of the substrate water molecules during the catalytic cycle, while D1Asp61 is strategically located at the mouth of a polar channel which probably functions to facilitate the exit of protons to the luminal surface. This channel is approximately 30 Å long and composed of side chains of the D1 protein (Asp61 and Glu65), D2 protein (Lys317 and Glu312) and the extrinsic PsbO protein (Asp158, Asp222, Asp223, Asp224, His228 and Glu 114). As well as acting as a pathway for removing protons, it probably also provides a route for supplying water molecules to the active site where both functions are aided by a Ca^{2+} bound at the luminal end of the channel ligated by PsbO residues (Murray & Barber 2006). Recently, a closer analysis of the Ferreira *et al.* structure (Murray & Barber 2007) indicated two additional channels leading from the OEC to the

luminal surface. One is sufficiently polar to also act as a H^+ /water channel while the less polar nature of the other suggests that it may promote rapid oxygen diffusion from the catalytic site.

The work of Ferreira *et al.* (2004) also established that D1His190 was in hydrogen-bonding distance to D1Tyr161 as required, and predicted, for the oxidation of the latter by the primary electron donor of PSII, P680, to generate the neutral tyrosine radical (Hoganson & Babcock 1997).

The model proposed by Ferreira *et al.* (2004) has recently been analysed in considerable depth using QM/MM analysis (Sproviero *et al.* 2006, 2007). The calculations assumed that the carbonate, tentatively identified in the X-ray structure, is replaced by a chloride ion in the active S_1 -state of the water-splitting catalytic cycle and that the assigned protein ligands were complemented by water and hydroxyl ligands to satisfy the coordination requirements of the five metal ions. Even with these adjustments, the calculated model for the metal cluster was remarkably similar to that proposed by Ferreira *et al.* (2004) and confirmed that it is a chemically stable structure even in the absence of protein ligands. Nevertheless, despite the good correlation between the Ferreira *et al.* structural model of the OEC and theoretical calculations, there are inconsistencies with distance and angular information derived from EXAFS. Indeed, recent polarized EXAFS studies conducted on single crystals of PSII isolated from *T. elongatus*, give at least four different arrangements for the $\text{Mn}_4\text{Ca}^{2+}$ cluster (Yano *et al.* 2006) while a more recent crystal structure (Loll *et al.* 2005) suggests yet another organization. Radiation damage during the collection of X-ray diffraction data has been implied as being the cause for inconsistencies between the different models (Yano *et al.* 2005). Recently, Barber & Murray (2008) have attempted to rationalize the existing data to provide a series of working models of the OEC. The differences between them are not large, having the same amino acid environments as first defined by Ferreira *et al.* (2004). Figure 1c shows one such model in which Mn4 is linked to the Mn_3CaO_4 cubane via one of its Mn (Mn3) rather than by a bridging oxygen of the cubane. As a consequence, this new arrangement has one rather than two mono- μ -oxo bonds and is more compatible with EXAFS analyses (Yachandra 2002). The adjustment of the linkage between Mn4 and the cubane requires a repositioning of the other Mn ions and therefore changes in the amino acid ligation pattern as emphasized by comparing figure 1b,d.

4. OXYGEN-EVOLVING MECHANISM

Although the precise geometry of the Mn_4Ca cluster and its exact ligand field characteristics are not yet known precisely, either for its relaxed S_1 -state or for higher S -state conditions, the models available do provide a basis for developing chemical mechanisms for the water oxidation and dioxygen formation. The location of one Mn ion (Mn4 or dangler Mn) adjacent to the Ca^{2+} and their positioning towards the side chains of several key amino acids, including the redox active TyrZ, suggests that they provide the ‘catalytic’ surface for binding the two substrate water molecules

Figure 2. (a,b) Possible mechanisms for the formation of dioxygen during the S4-S0 transition.

and their subsequent oxidation. One well-championed mechanism (Messinger *et al.* 1995; Pecoraro *et al.* 1998; Messinger 2004; McEvoy & Brudvig 2004, 2006) suggests that the substrate water, associated with Mn4, is deprotonated during the S-state cycle. This mechanism is dependent on Mn4 being converted to a high-oxidation state (possible Mn(V)) during progression to the S4-state just prior to O–O bond formation. The other three Mn ions are also driven into high-valency states (Mn(IV)) by S4 and act as an oxidizing battery for the oxo-Mn4 complex. In this way, the oxo is highly electrophilic, so much so that it makes an ideal target for a nucleophilic attack by the oxygen of the second substrate water bound within the coordination sphere of the Ca²⁺ (figure 2). An alternative mechanism suggests that the deprotonated water molecule on Mn4 forms a radical and that this attacks an oxygen atom linking Ca²⁺ with an Mn (Siegbahn 2006) or the oxygen of a water molecule coordinated to the Ca²⁺ to form the O–O bond.

5. EVOLUTIONARY ORIGIN OF PSII AND THE OEC

The evolutionary path that gave rise to the OEC of PSII remains a mystery. Despite this there are several features of PSII that link it back to anaerobic photosynthesis.

(a) Reaction centres

When the genes for the L and M subunits of the RC of purple photosynthetic bacteria were first sequenced, it became immediately clear that they were homologous to those which encode the D1 and D2 proteins of PSII (Youvan *et al.* 1984; Williams *et al.* 1986; Barber 1987; Michel & Deisenhofer 1988). This remarkable similarity of PSII reaction centres and their bacterial counterpart has been reinforced over the years and has led to their classification as type II RCs. X-ray crystallography has shown that the arrangement of cofactors on the acceptor side is essentially identical in the aerobic and anaerobic RCs (figure 3), which is also the case for their transmembrane helices as indicated in figure 4. The main differences between

them are found on the donor side with different cofactors and more extensive luminal hydrophilic domains for the D1 and D2 proteins compared with the L and M subunits. As detailed above, in the case of the D1 protein, the C-terminal domain together with the loop joining transmembrane helices C and D contains most of amino acids that constitute the OEC. The structural relationship between the L/M and D1/D2 proteins also carries over to the C-terminal domains of the reaction-centre proteins of photosystem I (PSI) and almost certainly to that of the strictly anaerobic green sulphur photosynthetic bacteria despite the fact these type I RCs have a FeS centre as their terminal electron acceptor (Rhee *et al.* 1998; Schubert *et al.* 1998; Murray *et al.* 2006). Therefore, there is no doubt that the reaction centres of all types of photosynthetic organisms present today evolved from a common ancestor. The fact that both types 1 and 2 consist of two homologous proteins (identical in the case of green sulphur photosynthetic bacteria) suggests that the common ancestor was derived from gene duplication.

(b) Inner light-harvesting systems

The two chlorophyll-binding proteins CP43 and CP47 each contain six transmembrane helices and, like those of the D1 and D2 proteins, are related by the pseudo-twofold axis which relate the cofactors involved in primary and secondary electron transfer. Again, there is a remarkable structural similarity between them and the PSI reaction-centre proteins, this time at their N-terminus (Rhee *et al.* 1998; Schubert *et al.* 1998; Murray *et al.* 2006; see figure 5). As shown in figure 6a, the most striking difference being the presence of the large loop joining helices V and VI in the case of the PSII proteins, which for CP43 contains amino acids, that makes up a part of the OEC as mentioned above. The large loop of CP47 also contains a conserved region consisting of bulky side chains including several phenylalanines occupying a cavity that is symmetrically related to the OEC (Ferreira *et al.* 2004). Crystallography (Ferreira *et al.* 2004; Murray *et al.* 2006) has shown that many of the Chl-binding sites in CP47 and CP43 are conserved in the PSI RC proteins (figure 6b). Curiously, this basic six transmembrane Chl-binding helical protein unit is not found in purple photosynthetic bacteria but its probable existence in the RC of anaerobic green sulphur bacteria indicates that it has a long evolutionary origin. Moreover, it is the basis of the Pcb protein of prochlorophytes and of the iron-stressed-induced (IsiA) protein of cyanobacteria (Chen *et al.* 2005; Murray *et al.* 2006) and clearly is a basic building block found in most photosynthetic organisms.

(c) The extrinsic proteins of the OEC

As far as we know, the components of the OEC, which are located in membrane-spanning proteins, are highly conserved across the complete range of oxygenic photosynthetic organisms. All the amino acids identified in the D1, D2 and CP43 proteins as being functionally important for the water-splitting reaction are conserved in all genomes sequenced to date. Also, the PsbO protein is ubiquitous to PSII in all types of oxygenic photosynthetic organisms. By contrast, the PsbV and PsbU extrinsic proteins of the OEC of cyanobacteria are not

Figure 3. Comparison of the electron transfer cofactors in the (a) reaction centres of photosynthetic purple bacteria (from *Rhodospseudomonas viridis*; Deisenhofer *et al.* 1985) and (b) photosystem II (from *T. elongatus*; Ferreira *et al.* 2004) emphasizing the similarity on the electron acceptor side for both systems but not on the donor side. The red arrows show the electron transfer pathways. Haem is one of the four haems of the cytochrome donor; BChl and Chl are bacteriochlorophyll and chlorophyll, respectively; BPheo and Pheo are bacteriopheophytin and pheophytin, respectively; L and M refer to L and M subunits, respectively; D1 and D2 refer to D1 and D2 proteins, respectively; MQ, menaquinone; UQ, ubiquinone; PQ, plastoquinone; Fe, non-haem iron. TyrZ and TyrD are redox active D1Tyr161 and D2Tyr160, respectively.

Figure 4. Comparison of structures (side views) of the (a) RC of purple photosynthetic bacteria (from *Rhodospseudomonas viridis*; Deisenhofer *et al.* 1985), (b) the D1 and D2 proteins (from *T. elongatus*; Ferreira *et al.* 2004) and (c) PSII monomer core with its 19 different subunits (from *T. elongatus*; Ferreira *et al.* 2004).

found in higher plants or green algae (red algae contain PsbV). Instead, the latter contain the PsbP and PsbQ proteins that show little or no homology with PsbV and PsbU. However, PsbP- and PsbQ-like proteins seem to exist in cyanobacteria (Kashino *et al.* 2002) although as yet they have not been present in isolated PSII complexes used for crystallography.

In principle, these extrinsic OEC proteins should give hints as to the evolutionary origin of the OEC. However, detailed analyses have not revealed any indications (De Las Rivas & Barber 2004; De Las Rivas *et al.* 2004). The PsbV protein of cyanobacteria is a low-potential cytochrome (*c*-type) with no active role in the catalytic activity of the OEC. It therefore seems to be an evolutionary relic of an electron transport system comparable with the donor side of purple bacterial reaction centres. It is clear, however, from crystallography (Ferreira *et al.* 2004; Loll *et al.* 2005) and model building based on EM (Morris *et al.* 1997; Nield & Barber 2006) that these extrinsic proteins form a 'cap' over the OEC and therefore prevent reductants other than water reacting with the catalytic centre.

Since the PsbO protein is ubiquitous to all types of oxygenic photosynthetic organisms, it should be the

best candidate for tracing the evolutionary origin of the OEC. The main body of the protein is a β -barrel composed of eight antiparallel β -strands. A very large loop joining β -strands 1 and 2 provides a head domain, which binds to the luminal surfaces of the D1, D2, CP43 and CP47 proteins. The β -barrel is not hollow but full of bulky side chains. A search of the database revealed a few proteins with structural homology with the β -barrel. Of these, quinoxaemoprotein amine dehydrogenase (QHNDH; pdb 1jju-A1) being perhaps the most interesting. This bacterial enzyme is an amine-quinone oxidoreductase in bacteria and has a β -barrel as one of its three subunits. Interestingly, it also contains two Cyt *c* haems. It is a matter of debate whether PsbO is a relic of this amine oxidase system.

(d) Cytochrome b559

Cytochrome b559 is a universal component of PSII. It is characterized by having a high redox potential with the ability to protect PSII reaction centres from oxidation by acting as an electron donor to P^{+} when the rate of water oxidation is limiting. The haem is ligated by two coaxial histidines contained in the apoproteins, PsbE (α -subunit) and PsbF (β -subunit).

Figure 5. Overlay of the structures of (a) carbon backbones of CP43 (yellow), CP47 (brown), PsaA (green) and PsaB (blue) and of (b) the conserved 12 Chls of CP43 (yellow), CP47 (purple), PsaA (green) and PsaB based on the crystal structures of PSII (Ferreira *et al.* 2004) and PSI (Jordan *et al.* 2001), where suffix C and N indicate C- and N-terminal domains.

Figure 6. Top stromal views derived from X-ray crystallography of (a) PSII (Ferreira *et al.* 2004) and (b) PSI (Jordan *et al.* 2001) to emphasize the similarity in the organization of transmembrane helices of CP43/D1 and CP47/D2 with those of PsaA and PsaB.

Despite having an unusual structure and high redox potential, its evolutionary origin is not obvious.

(e) The Mn_4Ca cluster

There are a number of enzymes that contain one or two Mn ions at their catalytic centres but the cluster of four found in the OEC is unique. Blankenship & Hartman

(1998) argued that perhaps the OEC derived from a two-Mn cluster of the type found in catalase which would be expected to give a symmetrical arrangement something like the dimer-of-dimer model favoured from earlier EXAFS analyses (see Yachandra 2002). However, the Mn_3CaO_4 cubane organization with a dangler Mn attached to it does not fit comfortably with

this hypothesis although the idea that PSII evolved from a hydrogen peroxide catalase is interesting. Russell & Hall (2001) proposed the OEC may have evolved from manganese oxide precipitates similar to rancieite ($\text{Mn}_4\text{CaO}_9 \cdot 3\text{H}_2\text{O}$) an idea that has been explored in depth by Sauer & Yachandra (2002).

The presence of five metal ions in a single catalytic centre is also very unusual. However, there is one interesting example, carbon monoxide dehydrogenase found in anaerobic bacteria such as *Carboxydotherrnus hydrogenoformans* (Dobbek *et al.* 2001). This enzyme contains four Fe ions and a Ni ion with S bridges. The arrangement of the ions is remarkably like those proposed for the metal centre of the OEC, a Fe_3Ni cubane with the fourth Fe ion linked to the cubane as a dangler. The enzyme catalyses the water shift reaction to generate reducing equivalents from water

The mechanism for the oxygen atom transfer involves CO bound to Ni and the substrate water to the dangler Fe. The similarities between this reaction and the water oxidation reaction of PSII and between the geometries of the catalytic centres are interesting but it seems unlikely that there is a direct evolutionary link. Nevertheless, carbon monoxide could have played a major role in bioenergetics prior to the evolution of the OEC (Sleep & Bird 2007). A plentiful supply of CO could have come from the photochemically (UV) driven disproportionation reaction between methane and carbon dioxide.

6. CONCLUSION

X-ray crystallography together with a wide range of biophysical, biochemical and molecular biological techniques has provided much detail of the molecular properties of PSII. In so doing, we are very close to revealing the precise chemical mechanism of the water-splitting reaction by which photosynthetic organisms generated the oxygenic atmosphere of our planet. The reaction is powered by light-driven charge separation across the RC of PSII and it is clear that this RC evolved from the same ancestor as the RC of PSI, purple photosynthetic bacteria and green sulphur bacteria. Moreover, the six transmembrane helical chlorophyll-binding unit typified by CP43 and CP47 is also found in PSI and green sulphur photosynthetic bacteria, again providing evidence of a common evolutionary origin for anaerobic and aerobic photosynthesis. At present, however, there are no obvious indications of the evolutionary origin of the OEC. One clear message, however, is that based on current knowledge it seems that the catalytic site of the OEC is fully conserved in all types of oxygenic photoautotrophs. Therefore, we can assume that the reaction giving rise to molecular oxygen in the atmosphere was invented only once and that the structural characteristics of its catalytic centre and its mechanism have been conserved since its conception.

I particularly wish to thank Dr James Murray and Dr Javier De Las Rivas for their contributions to the analyses of various aspects of PSII structure and referred to in the paper. I also acknowledge financial support from the Biotechnology and Biological Research Council (BBSRC).

ENDNOTE

¹In fact, in the case of the unusual cyanobacterium *Acaryochloris marina*, Chl *a* is replaced almost entirely by Chl *d* (Miyashita *et al.* 1996).

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