Photosynthetic Generation of Oxygen
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The oxygen in the atmosphere is derived from the 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.

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

Some where in the region of 2.5 billion years 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.

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2H₂O \rightarrow_{4\text{hv}} O₂ + 4e + 4H^+.
4e + 4H^+ + CO₂ \rightarrow (CH₂O) + H₂O
\]

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 twenty 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 fully exploit the terrestrial environment. In a nutshell, when biology learnt to split water using sunlight it simply 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, Wydrynski & Satoh 2005). In contrast to chemical and electrochemical water splitting, which are thermodynamically highly demanding, the PSII-catalyzed 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 Wydrynski & Satoh (2005)). These processes are initiated through the absorption of light energy by the 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 the CP43 and CP47 proteins. In total there are about 36 Chl a and 11 β-carotene per PSII core based on biochemical (Barbato et al 1991) and structural analyses (Zouni et al 2001, Kamiya & Shen 2003, Ferreira et al 2004, Loll et al 2005) (but see Footnote).

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, denoted 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•+Pheo•-. Within a few hundred picoseconds, Pheo•- reduces a firmly bound plastoquinone molecule protein (QA) to produce P•+PheoQA•-

Footnote: In fact in the case of the unusual cyanobacterium Acaryochloris marina, Chl a is replaced almost entirely by Chl d (Miyashita et al 1996).
The radical cation, \( \text{P}^{+} \), has a very high redox potential (>1V) and oxidises a tyrosine residue (Tyr\(_Z\)) to form Tyr\(_Z\)•PPheoQ\(_{A}^-\) on a nanosecond time scale. The oxidation of Tyr\(_Z\)• is dependent on the deprotonation of its phenolic group to generate a neutral radical (Tyr\(_Z^\cdot\)). In the millisecond time domain Q\(_{A}^-\) reduces a second plastoquinone (Q\(_B\)) to form Tyr•PPheoQ\(_{A}Q_{B}^-\). At about the same time Tyr• extracts an electron from a cluster of four manganese ions and a calcium ion (Mn\(_4\)Ca-cluster) that binds two substrate water molecules. A second photochemical turnover reduces Q\(_B^-\) to Q\(_B^{2-}\) which is then protonated to plastoquinol and released from PSII into the lipid bilayer where it is available to be oxidised by photosystem I (PSI) via the cytochrome b\(_{6}\)f complex. Two further photochemical turnovers provide the four oxidising equivalents required to oxidise two water molecules to form 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 (S\(_0\) to S\(_4\)). 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 \( \beta \)-carotene molecule and a Chl \( \alpha \) molecule (Chl\(_Z\)) (Tracewell & Brudvig 2003, Faller et al 2001, Telfer 2002, Stewart & Brudvig 1998). 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 PSIIRC core complex of plants and cyanobacteria was dimeric (reviewed in Hankamer et al 1997). In fact the early EM studies of my research group, 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 which 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 helices 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 (about 7 Å in width). Although the EM studies had provided the first glimpse of the
structural organisation 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 crystals 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 3D crystals and obtain a structural model at a resolution of 3.8 Å. However in this initial study the tracing of C-alpha backbones of some subunits was not completed and amino acids were not assigned, other than a tentative identification of D1Tyr161 (Ty\( \text{r}_Z \)) and D2Tyr160 (Ty\( \text{r}_D \)). 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-two-fold axis relating the transmembrane helices of the D1- and D2- reaction centre 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-two-fold symmetry of the PSII reaction centre and therefore placed it in the vicinity of Ty\( \text{r}_Z \) and close to the surface helix located in the loop joining the luminal ends of the C- and D-transmembrane helix 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 organisation 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 by Hasegawa et al (1999), and was a feature of the crystallographic model of the Mn-cluster derived by Kamiya and 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, CP43 and CP47. The positioning of cofactors was essentially the same as that reported by Zouni et al (2001). Kamiya and 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 and Shen’s map contained electron density connecting to that of the Mn-cluster tentatively assigned to side chains of the D1-protein including those previously identified by site directed mutagenesis (Diner 2001, Debus 2001).
As in the case of the earlier crystal structure (Zouni et al 2001), the model of Kamiya and Shen did not include a Ca\(^{2+}\) bound close to the Mn-cluster.

A breakthrough came in 2004 with the publication of the first complete and refined structure of PSII (Ferreira et al 2004) where over 5000 amino acids were assigned in the dimeric complex. Consequently this 3.5 Å model identified and gave the structures of 19 different subunits of the \(T.\) elongatus PSII complex, except for one low molecular weight intrinsic subunit tentatively assigned to \(\text{PsbN,}\) which is almost certainly \(\text{PsbYcf12}\) (Kashino et al 2007). 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 metal in the small domain and three in the large globular domain, whereas the Ca\(^{2+}\) anomalous difference map suggested Ca\(^{2+}\) in 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 (see Fig. 1A).

The much improved quality of the electron density map obtained by 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\(_4\)Ca-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 Mn₄Ca-cluster (Fig.1A/B). However, at the resolution of the diffraction data, the precise positioning of the metal ions could not be determined. Nevertheless, calculations using Density Function Theory (Lundberg & Siegbahn 2004), state-of-the-art Quantum Mechanic/Molecular Mechanic (QM/MM) methodologies (Sproviero et al 2006) and chemical synthesis of a mixed Mn/Ca complex (Misra et al 2005) indicated that the model suggested by Ferreira et al (2004) is 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 and Lundberg 2005, Siegbahn 2006). Based on this organisation of the metal ions, the Mn₃CaO₄ cubane had four protein side chains as ligands: D1Asp342 to Mn1, D1Glu189 and D1His332 for Mn2 and CP43Glu354 for Mn3 (see Fig.1A/B). Identification of the glutamate of CP43 as a 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 3₁₀ helix in the large extrinsic loop joining the luminal ends of transmembrane 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 Ca²⁺ 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 function as a ligand via a bridging water molecule. Because the coordination number is usually six or seven for Ca²⁺ and five or six for Mn, then according to the Ferreira et al model (Fig A/D) there would need to be additional non-protein ligands present, such as water molecules or hydroxides. Nevertheless Ferreira et al (2004) did emphasise that there was non-protein electron density in the vicinity of Mn(4) and Ca²⁺ which they tentatively assigned to a carbonate ion that formed bridging ligands between the two metals. The positioning of this carbonate ion adjacent to Tyr₂ 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 about 30 Å long and composed of side chains of the D1 protein (Asp61, Glu65), D2 protein (Lys317, Glu312) and the extrinsic PsbO protein (Asp158, Asp222, Asp223, Asp224, His228 and Glu114). 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 Ca²⁺ bound at the luminal end of the channel ligated by PsbO residues (Murray and Barber 2006). Recently, a closer analysis of the Ferreira et al structure (Murray and Barber, 2007) indicated two additional channels
leading from the OEC to the lumenal surface; one 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(TyrZ) as required, and predicted, for the oxidation of the latter by P•+ to generate the neutral tyrosine radical (Tyr•) (Hoganson and Babcock 1997).

As mentioned above, the model proposed by Ferreira et al (2004) has recently been analysed in considerable depth using QM/MM analysis (Sproviero et al 2006, 2007a,b). The calculations assumed that the carbonate, tentatively identified in the X-ray structure, is replaced by a chloride ion in the active S1 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. Despite 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.

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 polarised EXAFS studies conducted on single crystals of PSII isolated from T. elongatus, give at least four different arrangements for the Mn4-Ca2+-cluster while a more recently determined crystal structure (Loll et al 2005) suggests yet another organisation. 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 2006). Recently Barber and Murray (2007) have attempted to rationalise 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). Fig. 1C/D shows one such model in which Mn4 is linked to the Mn3CaO4-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 emphasised by comparing Figs. 1A/B with Fig. 1C/D.

4. OXYGEN EVOLVING MECHANISM

Although the precise geometry of the Mn4 Ca-cluster and its exact ligand field characteristics are not yet known precisely, either for its relaxed S1-state or for higher S-state conditions, the models available do provide a basis for developing chemical mechanisms for 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 Tyr\(_Z\) suggests that they provide the ‘catalytic surface’ for the binding of two substrate water molecules and their subsequent oxidation. One well championed mechanism (Messinger et al 1995, Pecoraro et al 1998, Messinger 2004, McEvoy & Brudvig 2004, 2006, Brudvig 2007) suggests that the substrate water associated with Mn4 is deprotonated during the S-state cycle and is converted to a high oxidation state (possible Mn(V)) during progression to the S\(_4\)-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 ‘oxidising 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 Ca\(^{2+}\) (see Fig. 2A). An alternative mechanism suggests that the deprotonated water molecule on Mn4 forms a radical and that this attacks an oxygen atom linking Ca\(^{2+}\) with a Mn (Siegahahn 2006, 2007) or the oxygen of a water molecule coordinated to the Ca\(^{2+}\) to form the O-O bond (Sproviero et al 2007a,b) (see Fig 2B). For an in depth discussion of these mechanisms see Betley et al (2007).

**5. EVOLUTIONARY ORIGIN OF PSII AND THE OEC**

The evolutionary path which 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 reaction centre of purple photosynthetic bacteria were first sequenced it became immediately clear that they were homologous with those that encode the D1 and D2 proteins of PSII (Youvan et al 1984, Williams et al 1986, Barber 1987, Michel & Diesenhofer 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 (see Fig 3), which is also the case for their transmembrane helices (see Fig 4). The main differences between them are found on the donor side with different cofactors required for the water splitting reaction in the PSII RC and associated extensions of the loops joining the transmembrane helices and of the C-terminal domains of the D1 and D2 proteins, as compared to the L and M subunits.

Fig. 3 (Left). Comparison of the electron transfer cofactors in the reaction centres of photosynthetic purple bacteria (from *Rhodopseudomonas viridis*–Diesenhofer et al 1985) and photosystem II (from *Thermosynechococcus elongatus*–Ferreira et al 2004) emphasising 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 pheophyin respectively, L and M refer to L-subunit and M-subunits; D1 and D2 refer to D1 and D2 proteins. MQ, menoquinone; UQ, ubiquinone; PQ, plastoquinone; Fe, non-haem iron. TyrZ and TyrD are redox active D1Tyr161 and D2Try160 respectively.

Fig. 4 (Right). Comparison of structures (side views) of the reaction centre (RC) of purple photosynthetic bacteria (from *Rhodopseudomonas viridis*–Diesenhofer et al 1985), the D1 and D2 proteins (from *Thermosynechococcus elongatus*–Ferreira et al 2004) and PSII monomer core with its 19 different subunits (from *Thermosynechococcus elongatus*–Ferreira et al 2004).

As detailed above, in the case of the D1 protein, the C-terminal domain together with the loop joining transmembrane helices C and D, contain most of the 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 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) (see Fig 6). Therefore there is no doubt that the reaction centres of all types of photosynthetic organisms present today evolved from a common ancestor (Rhee et al 1998, Schubert et al 1998). The fact that both Type I and II consist of two homologous proteins (identical in the case of green sulphur 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-two fold
axis relating 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 Figs 5 and 6). The most striking difference being the presence of the large loops joining the luminal ends of helices V and VI of the PSII proteins, where some amino acids of the CP43 loop make up a part of the OEC as mentioned above. The large loop of CP47 also has a conserved region containing bulky side chains including several phenylalanines which occupy a cavity symmetrically related to the OEC (Ferreira et al 2004).

![Fig. 5(Right). 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).](image)

As emphasised in Fig 5B, X-ray crystallography (Jordan et al 2001, 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. 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 suggests that it has a long evolutionary origin. Moreover it is the basis of the Pc-b-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. In contrast the PsbV and PsbU extrinsic proteins of the OEC of cyanobacteria are not found in higher plants or green algae (red algae contain PsbV). Instead the latter contain the PsbP and PsbQ proteins which show little or no homology with PsbV and PsbU. However PsbP-like 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 to this effect (De Las Rivas et al 2004, De Las Rivas & Barber 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 electron microscopy (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 back 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 provide a head domain which binds to the lumenal 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 data base revealed a few proteins with structural homology with the β-barrel. Of these the quinohaemoprotein 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 oxidase system.

(e) Cytochrome b559. Cyt b559 is a universal component of PSII. It is characterised by having a high redox potential (~0.4V) 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 co-axial histidines contained in the apoproteins, PsbE (α-subunit) and PsbF (β-subunit). Despite having an unusual structure and high redox potential its evolutionary origin is not obvious.

(f) The Mn₄Ca-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 and Hartman (1998) argued that perhaps the OEC was
derived from the duplication of 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$_3$CaO$_4$ cubane organisation with a dangler Mn attached to it does not fit comfortably with this hypothesis although the idea that PSII evolved from a hydrogen peroxide oxidase is interesting. Russell and Hall (2001) proposed the OEC may have evolved from precipitates of manganese oxides similar to rancieite (Mn$_4$CaO$_9$.3H$_2$O) an idea which has been explored in depth by Sauer and 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 Carboxdothermus hydrogenoforman (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 by Ferreira et al (2004), a Fe$_3$Ni-cubane with the fourth Fe ion linked to the cubane as a “dangler.” The enzyme catalyses the water shift reaction to generate protons (H$^+$) and electrons (e) from water

$$\text{CO} + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{H}^+ + 2\text{e}.$$ 

The mechanism for the oxygen atom transfer involves CO bound to Ni with the substrate water probably associated with the “dangler” Fe. The similarities between this reaction and the water oxidation reaction of PSII and between the geometries of the catalytic centres is 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.

$$\text{CH}_4 + \text{CO}_2 \rightarrow \text{CO} + \text{H}_2$$

6. CONCLUSION

X-ray crystallography together with a wide range of biophysical, biochemical and molecular biological techniques have 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 for the RC of PSI, purple photosynthetic bacteria and green sulphur bacteria. Moreover the six transmembrane helical chlorophyll-binding unit typified by the CP43 and CP47 is also found in PSI and green sulphur bacteria, again providing evidence of a common evolutionary origin for anaerobic and
aerobic photosynthesis. At present, however, there is no obvious indication 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 mechanism have been conserved since its conception.

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