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# Photosystem II and photosynthetic oxidation of water: an overview

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Conceptually, photosystem II, the oxygen-evolving enzyme, can be divided into two parts: the photochemical part and the catalytic part. The photochemical part contains the ultra-fast and ultra-efficient light-induced charge separation and stabilization steps that occur when light is absorbed by chlorophyll. The catalytic part, where water is oxidized, involves a cluster of Mn ions close to a redox-active tyrosine residue. Our current understanding of the catalytic mechanism is mainly based on spectroscopic studies. Here, we present an overview of the current state of knowledge of photosystem II, attempting to delineate the open questions and the directions of current research.

**Keywords:** photosynthesis; water oxidation; oxygen evolution; manganese enzyme; photosynthetic reaction centre

## 1. INTRODUCTION

The oxidation of water, using the energy from visible light, is a clever trick of Nature and one that scientists would like to understand and master. Oxygenic photosynthetic species seem to have been performing this trick for about three billion years and it has served them well. The success of plants, algae and cyanobacteria has been a key factor for determining the nature of life on Earth, with photosynthesis being responsible not only for energy input to the biosphere but also for $O_2$ input and $CO_2$ uptake to and from the atmosphere.

The enzyme responsible for water oxidation and oxygen evolution is at the start of the photosynthetic electron transfer chain, where it uses light energy to generate sufficient oxidizing power to extract electrons from water, thus providing the system with a source of electrons that is essentially unlimited, while delivering electrons to plastoquinone. The enzyme can therefore be considered as a water/plastoquinone photo-oxidoreductase and it is referred to as PSII.

In this review, we shall provide a brief overview of the current state of knowledge of this enzyme. For more detailed reviews, the interested reader is directed elsewhere (Debus 1992; Britt 1996; Hoganson & Babcock 2000; Robblee *et al*. 2001; Aznar & Britt 2002; Vrettos & Brudvig 2002; Yachandra 2002).

## 2. THE PHOTOCHEMICAL REACTION CENTRE

The PSII reaction centre is made up of a complex of pigment-bearing proteins that are embedded in a membrane (figure 1). The complex, which has a mass of *ca*. 300 kDa, is made up of many (greater than 25) protein subunits and at least 13 redox-active cofactors (see Hankamer *et al.* 1997). The electron-transfer events are restricted to the major proteins of the central core. The most important subunits are a pair of intertwined proteins with molecular masses of *ca*. 30 kDa, which are known as D1 and D2 (D for 'diffuse' bands on polyacrylamide gels). These two proteins contain all the cofactors involved directly in water oxidation and plastoquinone reduction. The D1 subunit contains the Mn cluster that constitutes the site of water oxidation. D1 and D2 are very similar to each other and are homologous to the L and M proteins in bacterial reaction centres (Michel & Deisenhofer 1988) and, as such, make up a heterodimer that has probably evolved from what was once a homodimeric system (Rutherford & Nitschke 1996). The cofactors are arranged symmetrically in this central core with one side making up the trans-membrane electron-transfer pathway.

The heterodimeric core is surrounded by two symmetrically arranged proteins that contain chlorophylls, which play a light collection role. These are the 43 and 47 kDa polypeptides, structurally homologous to the peripheral antenna part of the larger photosystem I-type reaction centres (Vermaas 1994; Rutherford & Nitschke 1996; Schubert *et al.* 1998; Barber *et al.* 1999; Zouni *et al.* 2001), but with additional large loops on the internal membrane surface. This electron-donor side-loop may simply serve to insulate the Mn cluster but it remains possible that the 47 kDa protein may also contribute to the binding site of the Mn cluster.

Among the many other proteins associated with the enzyme, one or two are worthy of mention even in this brief overview. Cytochrome *b*559 is made up of a protohaem and two small trans-membrane subunits (Stewart & Brudvig 1998). This cytochrome may be associated with processes involved in the assembly of the protein complex and perhaps the Mn cluster, and in protection of the reaction centre against oxidative damage, perhaps by an involvement in electron transfer around the reaction centre under certain circumstances. It has recently been

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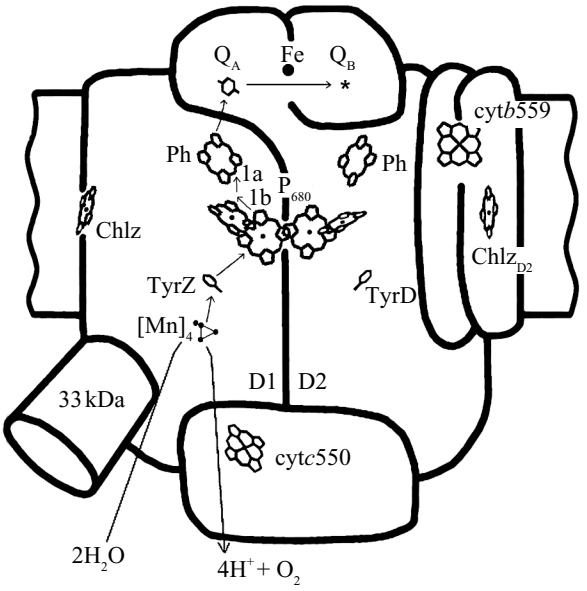


Figure 1. PSII and position of cofactors participating in the light-induced electron flow. (Modified from Zouni *et al.* 2001; see text for description.)

shown that electron donation from the cytochrome occurs via a redox-active carotene (Hanley *et al.* 1999) rather than via a redox-active chlorophyll, as previously thought.

On the lumenal (i.e. interior) surface of the enzyme, there are three extrinsic polypeptides, the 33, 23 and 17 kDa polypeptides that serve to insulate the catalytic site from reductive attack and to maintain $Ca^{2+}$ (23 kDa) and $Cl^-$ (33, 23 and 17 kDa) (Debus 1992). In cyanobacteria, the 23 and 17 kDa polypeptides seem to be replaced by other proteins, one of which is a low potential cytochrome *c*550 with no apparent redox role (Shen *et al.* 1998). Lastly, one small trans-membrane polypeptide of 4.4 kDa (the so-called PsbL subunit) seems to be necessary for TyrZ oxidation (Hoshida *et al.* 1997). From the current crystallographic model (Zouni *et al.* 2001), it seems that this effect must occur over quite a long distance, even if the trans-membrane helix closest to TyrZ is assigned to the L subunit (see Hankamer *et al.* 2001).

## 3. PHOTOCHEMISTRY

The main cofactors in the photochemical part of PSII are organized in a very similar way to those in the bacterial reaction centre (e.g. Rutherford 1989; Rhee *et al.* 1998; Zouni *et al.* 2001) except that the two central chlorophylls have a wider separation and are consequently more weakly coupled (Zouni *et al.* 2001; see also Svensson *et al.* 1996; Rutherford & Nitschke 1996; Dekker & van Grondelle 2000; Barber & Archer 2001). Taking the coupling energies in the bacterial reaction centre as a reference, it is clear that in PSII there is no dimer or 'special pair' of chlorophylls and all the pigments can be considered as monomers.

Charge separation is also comparable with that occurring in the bacterial reaction centre, with one or two significant differences. Absorption of a photon in PSII leads to the formation of an excited state of the chlorophyll species, $P^*$ (also known as $P^*_{680}$), a pigment complex that has its absorption maximum at 680 nm and seems to involve more than one (possibly all) of the core pigments (Dekker & van Grondelle 2000). The location of the excited state is thought to be predominantly on the chlorophyll closest to the active-side pheophytin (Diner *et al.* 2001). Charge separation probably takes place between this chlorophyll and the pheophytin (event labelled 1a in figure 1) (Rutherford 1988; Rutherford & Nitschke 1996; Dekker & van Grondelle 2000; Prokhorenko & Holzwarth 2000; Barber & Archer 2001; Diner *et al.* 2001). The first detectable charge pair comprises an oxidized chlorophyll cation and a pheophytin anion, $P^{\cdot+}Ph^{\cdot-}$, the cation being located on the chlorophyll nearest to TyrZ (the tyrosine 161 of the D1 protein) (Diner *et al.* 2001). This implies that a rapid electron transfer from this chlorophyll to the photo-oxidized chlorophyll takes place (event 1b in figure 1), but this has yet to be measured directly (Rutherford & Nitschke 1996; Dekker & van Grondelle 2000; Diner *et al.* 2001). Compared with the bacterial reaction centre, this sequence of events appears quite remarkable because the cofactor that is the primary electron acceptor in the bacterial system is the primary electron donor in PSII (Rutherford & Nitschke 1996). That said, to convert the arrowed scheme in figure 1 for primary electron transfer in PSII to a scheme applicable to the purple bacterial reaction centre, all that is required is to exchange the labels for events 1a and 1b.

Having formed the $P^{\cdot+}Ph^{\cdot-}$ radical pair, $Ph^{\cdot-}$ donates an electron in a few hundred picoseconds to a tightly bound quinone molecule, $Q_A^{\cdot-}$, forming the $P^{\cdot+}Q_A^{\cdot-}$ radical pair. $P^{\cdot+}$ is able to oxidize TyrZ. The kinetics of TyrZ formation vary from tens of microseconds to tens of nanoseconds depending on a range of factors including pH, the presence of $Ca^{2+}$ and the presence of the Mn cluster and its redox state. The TyrZ oxidation is accompanied by the loss of a proton, resulting in the formation of a neutral radical. Most indications show that, in the Mn-free system at least, the charge ($H^+$) stays close to the tyrosyl radical and is probably rebound upon reduction of the $TyrZ^{\cdot}$ (see § 17 for further discussion). The $TyrZ^{\cdot}Q_A^{\cdot-}$ radical pair is further stabilized by electron transfer from $Q_A^{\cdot-}$ to a second quinone, $Q_B$, and, in the functional enzyme, by electrons from the catalytic site, and ultimately from water (reviewed in Diner & Babcock 1996).

## 4. THE KINETIC MODEL FOR WATER OXIDATION

The reaction centre is a device for generating positive charge equivalents, with an oxidizing power of *ca.* 1.1 V in the form of a neutral tyrosyl radical (Diner & Babcock 1996; Tommos & Babcock 2000), and it works based on one oxidizing equivalent per photon. By contrast, the oxidation of water to form molecular oxygen is a four-electron redox reaction, $2H_2O \rightarrow O_2 + 4H^+ + 4e^-$. It is thus necessary to connect the univalent generator of oxidant to the device for extracting four electrons from water.

In principle, mechanisms could be imagined in which several reaction centres shared a single catalytic site. However, this kind of mechanism was ruled out by the classic work of Joliot and co-workers (Joliot *et al.* 1969), who used a rapidly responding polarographic oxygen electrode, and flashes of light bright enough to produce a photochemical charge separation in all the PSII reaction centres

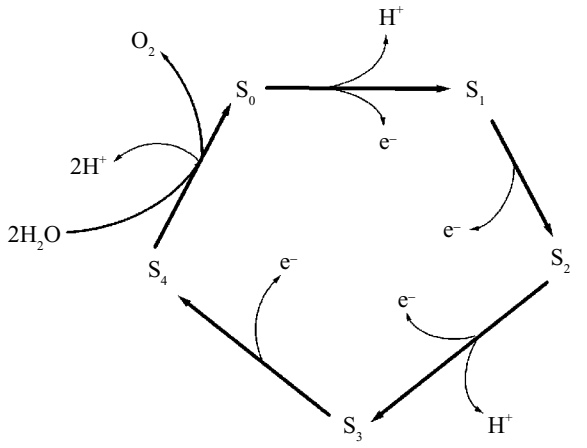


Figure 2. The S-state cycle model proposed by Kok (Kok *et al.* 1970).

in the sample, but short enough to do it only once. The resulting period of a four-oscillation pattern for oxygen release demonstrated that four photochemical turnovers were required for every molecule of $O_2$ that was released. Thus, each catalytic site was connected to a single photochemical reaction centre.

The features of the flash pattern were rationalized by Kok (Kok *et al.* 1970) within a kinetic model known as the S-state cycle. In this model, five states, designated $S_n$, of the enzyme are considered to exist, where $n$ is 0–4 and represents the number of positive charge equivalents accumulated. (The term 'charge equivalents' is used because reactions such as deprotonations etc. can occur at the active site, compensating for the accumulation of true positive charges.) When $S_4$ is generated it reacts rapidly (1 ms) releasing $O_2$ and returns to the reduced form of the enzyme $S_0$ (figure 2).

It transpires that the stable state of the enzyme in the dark is $S_1$, so on the first cycle only three photochemical turnovers are required before oxygen is released. The $S_2$ and $S_3$ states are unstable and relax back to the $S_1$ state in darkness, their lifetime being determined by the source of the electron. The lifetime is in the tens of seconds range when the electron comes from $Q_B^{\cdot-}$, through a route involving a luminescence-emitting back reaction, or a few minutes if the electron initially went further than $Q_B$ (Rutherford *et al.* 1982). The $S_0$ state is more stable, but is also converted to the $S_1$ state in darkness (Vermaas *et al.* 1984). This requires a strong oxidant and this role is played by the stable tyrosyl radical, $TyrD^{\cdot}$ that, although not kinetically competent in the catalytic reaction, will slowly oxidize $S_0$ to $S_1$ in darkness (Styring & Rutherford 1987). It has been suggested that this may be an advantage in terms of stability of the complex to be in $S_1$ rather than $S_0$. The TyrD is thought to oxidize the Mn through electron transfer equilibria between the intervening cofactors, i.e. TyrZ and the chlorophylls of P.

The kinetic model as postulated by Kok (Kok *et al.* 1970) has remained central in understanding the enzyme. The beauty of the S-state model is that it precisely describes the phenomenology without defining if a given step in the cycle represents the accumulation of a charge equivalent on a storage system, or it represents partial oxidation of the substrate. Since its conception, one of the main aims of research in this field has been to put some chemical meat on the abstract, S-state bones. This leads to the subject of manganese.

## 5. MANGANESE

A cluster of four Mn ions has a role in the charge accumulating process and very probably as the active site. The involvement of Mn with the oxygen-evolving enzyme has been known for many years. It is possible to remove the Mn from the enzyme and then, given the appropriate conditions, replace it again and regain activity. In fact, the PSII reaction centre is assembled in the membrane as a functional photochemical reaction centre without Mn and consequently without the ability to oxidize water. This is the case with several species (some green algae and plants such as spruce) when grown in darkness. The Mn cluster and the enzyme activity appear when the centres are illuminated (see Tamura & Cheniae 1987; Ananyev *et al.* 2001).

The assembly of the Mn complex requires Mn, $Ca^{2+}$, $Cl^-$, a functional PSII reaction centre (i.e. for *in vitro* studies, the addition of an artificial electron acceptor) and light. The light is required to generate the oxidant ($TyrZ^{\cdot}$) needed for oxidizing the $Mn^{II}$ ions to higher redox states. There is evidence for a sequential process involving binding and high quantum yield oxidation of $Mn^{II}$, a role for $Ca^{2+}$ binding, perhaps linked to folding changes in the protein, the binding and oxidation of a second $Mn^{II}$. This is followed by the association (and oxidation) of two more Mn ions, forming the final stable high valence $Mn_4$ cluster. Other than the first high quantum yield step, the redox events occurring in the assembly of the cluster are poorly defined because at least one of them occurs with a low quantum yield (see Tamura & Cheniae 1987; Ananyev *et al.* 2001).

## 6. STRUCTURE OF THE Mn CLUSTER

The structure of the Mn cluster is not known in detail, however, a range of spectroscopic methods have provided considerable information on the subject, much of it not without ambiguity. The main methods used have been EPR and X-ray absorption spectroscopy. Together they have provided information that has allowed limitations to be set on the possible structures and generated much debate. A poorly resolved X-ray structure has recently provided additional structural restraints and grounds for optimism that a detailed structure will be available in the not too distant future (Zouni *et al.* 2001).

## 7. EPR

One of the first, and strongest, indications that the S-state cycle involved the oxidation of Mn was the observation of an EPR signal upon formation of the $S_2$ state (Dismukes & Siderer 1981). This observation was probably the first good evidence for the involvement of a polynuclear Mn cluster. This so-called multiline EPR signal was centred at $g = 2$, arose from a spin equal to 1/2 ground state and showed many (greater than 18) resolved hyperfine lines attributable to at least two Mn nuclei. The signal

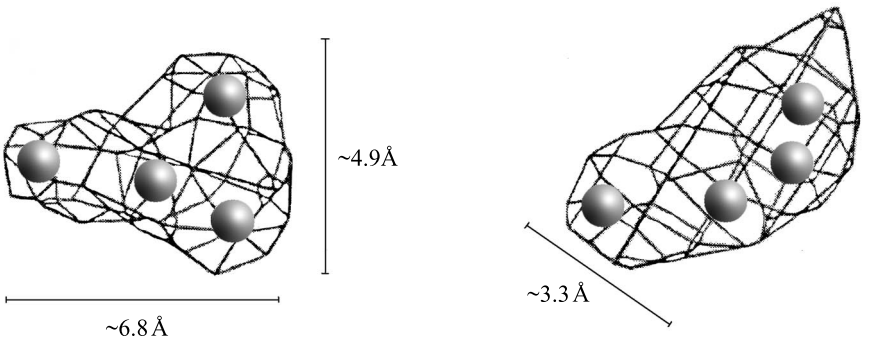


Figure 3. Illustration and approximate dimensions of the Y-shaped density map attributed to the Mn cluster of PSII. (Modified from Zouni *et al.* 2001.)

was similar to those seen in synthetic di-μ-oxo-bridged $Mn^{III}Mn^{IV}$ dimers. This EPR signal has been the focus of much research and has triggered great interest in the field of Mn cluster chemistry. In recent years, EPR signals arising from other S states or other spin states of the Mn in $S_2$ have been discovered (reviewed in Peloquin & Britt 2001). These signals have yet to provide much insight into the structural aspects of the Mn cluster and attention remains focused on the most studied $S_2$ multiline signal.

Structural insights from the $S_2$ multiline spectrum have been limited to a few basic points, and these are still disputed. It is best explained as a tetramer (Britt 1996; Carrell *et al.* 2001; Peloquin & Britt 2001). For some time thinking focused on the dimer of dimer models (influenced by the X-ray absorption models) from which the main features of the spectrum arose from a dominant coupling. This ranged from a di-μ-oxo dimer of $Mn^{III}Mn^{IV}$ with weaker couplings to two other Mn ions that are either both $Mn^{IV}$ or both $Mn^{III}$ ions (Zheng & Dismukes 1996; Blondin *et al.* 1997). In the past, other structures, such as distorted cubanes, butterflies and so-called 3 + 1 clusters (i.e. three tightly coupled and one weakly coupled Mn) were discussed. Recently, they have received renewed attention (Hasegawa *et al.* 1998; Peloquin & Britt 2001), especially with the benefit of the recent crystallographic model (Carrell *et al.* 2001) (see Zouni *et al.* 2001; figure 3).

Simulations of the spectra have provided rather ambiguous results. This is because the spectra provide insufficient information to allow a unique parameter set to be obtained from the fits. Additional restraints for simulations have been obtained from EPR spectra measured in oriented samples, at a range of frequencies and from several different forms of the signal (and related states) that can be generated in biochemically modified forms of the enzyme (Hasegawa *et al.* 1998; Carrell *et al.* 2001; Peloquin & Britt 2001; see also Åhrling *et al.* 1998). The measurement of Mn hyperfine couplings using pulsed electron nuclear double resonance has provided another important set of parameters for the simulations (Peloquin & Britt 2001). Other restrictions for the simulations have been obtained from studies of a model Mn cluster, from structural and valence information from X-ray absorption and, recently, the crystal structure. Overall, the recent simulation work has focused on clusters in which three Mn are relatively strongly coupled, while the fourth is more weakly coupled, i.e. 3 + 1 structures. Despite all these efforts, ambiguities remain; therefore, it is probably still true that structures derived from simulations should be considered as models that are compatible with the data rather than definitive structures.

### (a) *X-ray absorption*

Several structural models that have dominated the debate have come from EXAFS studies (Yachandra *et al.* 1996; Robblee *et al.* 2001). The method has provided information on the distance separating the Mn ions and through comparisons with model systems, the di-μ-oxo-bridged structures have been a central motif, at least for $S_1$ and $S_2$, the states most studied. Studies were taken as an indication of the existence of two such di-μ-oxo-bridged motifs and a so-called 'dimer-of-dimers' cluster was the favoured model for some years. EXAFS studies on oriented samples allowed further distinctions between the two 'dimers' and a series of topological structures consistent with the EXAFS data were delineated, one of which, the C-shaped dimer of dimers was the focus of particular attention. Recently, a shift of emphasis away from the C-shaped dimer of dimers towards another of the topologically consistent structures, a 3 + 1 structure, took place (Yachandra 2002).

### (b) *X-ray crystallography*

The 3 + 1-type tetramers recently favoured by EPR (Hasegawa *et al.* 1998; Peloquin *et al.* 2000) and X-ray spectroscopies occurred at a crucial stage as the recent crystallographic data (Zouni *et al.* 2001) seem to fit better with these than the earlier dimer-of-dimer models. The crystal structure although poorly resolved (3.8 Å) was able to detect the density from the metals in the protein; among these were the Mn ions. The data appear to fit with a cluster of three to four Mn as predicted from the spectroscopic studies with a preliminary Mn topography that has a distorted Y-shape (figure 3). A reassessment of the X-ray absorption spectroscopy, and in particular EPR data, has been carried out, taking into account the structural data from the crystal model. Coupling models and simulations have been presented based on a range of tetrameric structures with an emphasis on cubane and funnel structures (Carrell *et al.* 2001), although other 3 + 1 geometries (Peloquin *et al.* 1998) also appear to fit. Only minor improvements in the resolution of the crystal structure are required in order to remove the remaining ambiguity concerning Mn nuclearity and topography.

## 8. LOCATION OF THE Mn

The location of the Mn in the PSII protein complex remained the subject of debate, although most experimental indications pointed to a position on the internal side of the protein with ligands provided by D1 (Debus 1992). This was confirmed by the recently determined crystal structure (Zouni *et al.* 2001). The actual ligands have not yet been unambiguously identified although the current suspects include three histidines (190, 332 and 337), two aspartates (170 and 342), a glutamate (333) and the C-terminus alanine (344) (see Debus 2000, 2001; Diner 2001). A ligand, or ligands, from other proteins, perhaps CP47, have not yet been ruled out. Given the symmetry of the D1–D2 dimer, it is has been suggested that a second Mn site once existed in a symmetrical position associated with D2, but that this site has been lost through evolution (Rutherford & Nitschke 1996). The crystal structure places the Mn cluster at 7 Å edge to edge from the TyrZ (Zouni *et al.* 2001).

## 9. WATER CHEMISTRY

To understand the mechanism of water oxidation, several basic questions need to be answered: where, when and how? In what follows we shall attempt to bring the reader up to date with the situation concerning knowledge relevant to the 'where' and 'when' questions, both of which should provide tractable experimental problems. In principle, answers to these two questions should be helpful in answering the more difficult 'how' question.

Regarding the 'where' question, the good news is that we think we know that the water becomes oxidized when bound to the Mn cluster. The bad news is that we have no strong positive evidence for this. Rather than deal with a list of weak or ambiguous experimental observations, we shall quickly move on to the next question and, while dealing with that and subsequent sections on mechanisms, we shall point out some aspects that relate to the site of catalysis.

An important question that should be answerable experimentally is whether water is oxidized after the accumulation of all four of the charge equivalents (i.e. in $S_4$) or whether extraction of electrons from the substrate occurs at an earlier step (or steps) in the cycle? It should be possible to address this 'when' question by examining (i) the redox and protonation events occurring at each step and (ii) the substrate-binding properties. These aspects, the valence of the Mn, the roles of $Ca^{2+}$ and $Cl^{-}$ and a range of other studies, all contribute to some extent to the big question of how it works. Some of these themes are addressed below.

## 10. REDOX EVENTS DURING THE S-STATE CYCLE

If water oxidation occurs before the accumulation of four charge equivalents, then one or more of the steps in the cycle should correspond to no oxidation of cofactors (or possibly the reduction of cofactors in the case of redox chemistry involving more than one electron). What has been concluded, based on the Mn X-ray absorption edge, and (more ambiguously) on EPR and UV visible spectroscopies, is that the $S_0$–$S_1$ and the $S_1$–$S_2$ steps both correspond to one-electron oxidations of the Mn cluster (reviewed in Debus 1992). The $S_4$ state has not been resolved in spectroscopic studies but the oxygen-evolving step, the $S_3$–$S_0$ transition, gives spectroscopic changes indicating a return to the most reduced state (Debus 1992; Dekker 1992; Britt 1996). So far, so good. The problem arises for the $S_2$–$S_3$ state. For this transition, it is not clear what occurs.

It would be convenient if the Mn cluster underwent a straightforward oxidation when going to $S_3$, as it seems to do in the preceding steps. The disappearance of the $S_2$ EPR signals, the shift in the X-ray absorption edge reported by some groups and the majority of the UV visible data do favour this view (reviewed in Debus 1992; Dekker 1992; Britt 1996). Unfortunately, there are some experimental observations which force us to keep open the possibility that Mn oxidation does not occur on the $S_2$–$S_3$ transition.

(i) A number of X-ray absorption studies from the Berkeley group have consistently shown little or no change in the X-ray absorption edge on this step (Messinger 2000; Robblee *et al.* 2001). While this has been challenged (Iuzzolino *et al.* 1998), improved data and methods have continued to support the lack of a significant edge shift (Messinger *et al.* 2001). It is clear that the most straightforward interpretation of no change in the absorption edge is that it reflects no change in the valence. This has led to the proposal that a bridging μ-oxo radical is formed on this step instead of Mn oxidation (Messinger *et al.* 2001; Robblee *et al.* 2001). The marked lengthening of Mn–Mn distances upon $S_3$ formation has also been rationalized based on this model (see Robblee *et al.* 2001). Nevertheless, it remains possible that the absence of a shift in the X-ray absorption edge can be rationalized in terms of Mn oxidation actually taking place. For example, if the edge shift is rendered smaller than expected for other structural reasons, such as an unexpected quirk of the ligand sphere arising from the predicted conformational change (note that there are several lines of evidence other than X-ray spectroscopy that indicate a conformational change upon $S_3$ formation (Boussac & Rutherford 1988*a*; Messinger *et al.* 1991; Renger & Hanssum 1992).

(ii) EPR data showed that the non-integer spin states giving EPR signals in $S_2$ disappeared when $S_3$ was formed. This was assumed, reasonably enough, to indicate that oxidation of Mn had taken place. However, when the relaxation properties of TyrD were monitored during the S-state cycle, it was discovered that while the relaxation was slow for $S_1$ and fast for $S_2$ and $S_0$ (the states predicted to be paramagnetic), the $S_3$ state displayed relaxation properties similar to those of $S_2$. This was taken as an indication of no change in the redox state of the Mn cluster upon the $S_2$–$S_3$ step in accordance with the X-ray absorption edge data (Styring & Rutherford 1988). Nuclear magnetic resonance proton relaxation studies also showed no change on this transition (Sharp 1992). The disappearance of the $S_2$ EPR signals had to be rationalized by invoking an interaction with another

species, for example an oxidized radical species that interacts magnetically with the Mn cluster (Styring & Rutherford 1988). The idea was backed up by the discovery of exactly such a situation in the enzyme under inhibitory conditions. An amino acid radical was found to be in interaction with the Mn cluster in the $S_2$ state, formally, a modified $S_3$ state (Boussac *et al.* 1989). This supported the idea that $S_3$ could be a radical plus the Mn cluster in the $S_2$ redox state. The straightforward interpretation of no change in relaxation properties (as measured at the TyrD radical) was taken as no change in redox state of the Mn cluster. In this case, however, the phenomena are only indirectly related to Mn redox reactions: relaxation effects are poorly defined, and it is possible that a Mn oxidation upon $S_3$ formation could result in a state that coincidentally just happens to be as good as $S_2$ at relaxing $TyrD^{\cdot}$.

(iii) The unusual sensitivity of the $S_3$ state to reduction by NO was suggested to reflect the fact that it has a radical character (Ioannidis *et al.* 2000). Similarly, the surprising inertness of the $S_3$ state to reductive attack from amines and $OH^-$ compared with $S_2$ may be seen as an argument against an increase in the valence of the Mn cluster on this step (Messinger *et al.* 1991). The alternative explanation for these phenomena is that they reflect some kind of conformational change occurring on this step (Boussac & Rutherford 1988*a*; Messinger *et al.* 1991; Renger & Hanssum 1992; Robblee *et al.* 2001), thus changing the reactivity of the cluster.

It can be seen that all of the lines of argument against Mn oxidation on the $S_2$–$S_3$ transition are, to various degrees, ambiguous. EPR signals have recently been discovered from the $S_3$ state (Matsukawa *et al.* 1999; Ioannidis & Petrouleas 2000; Boussac *et al.* 2000), and we might expect them to contribute to the debate. These signals are detected using both conventional and parallel mode EPR (parallel mode EPR allows the detection of signals from integer spin states). The straightforward interpretation of an integer spin-state system from the Mn cluster would be that it is formed by oxidation of the non-integer spin state of $S_2$. If this is the case, this would resolve the controversy. Typically, however, ambiguities still creep in. The parallel mode signal could originate from a radical coupled to the Mn cluster in the $S_2$ redox state. Furthermore, it is not yet clear what proportion of centres contribute to $S_3$ EPR signals. Future studies on $S_3$ signals may resolve this argument.

Renger has championed $S_3$ as a state which could be a crypto-peroxide, a state where the substrate has undergone a two-electron oxidation existing in redox equilibrium with oxidized Mn with two bound water-substrate molecules (Renger 2001). The arguments are mainly from theoretical (thermodynamic and kinetic) considerations rather than from direct experimental evidence. Peroxide formation involves the two-electron oxidation of two molecules of water, therefore peroxide formation on the $S_2$–$S_3$ step should involve Mn reduction. There are no experimental indications for this and the substrate-binding work described in § 12 seems to argue against water oxidation before $S_4$ formation (see, however, Messinger 2000).

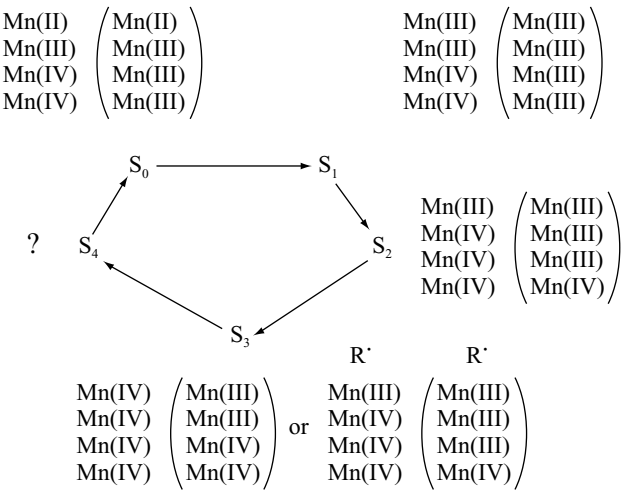


Figure 4. S-state model of the Mn cluster showing the current views on the valences of the four Mn ions of the tetramer. The parentheses show the low valence option. See text for a discussion.

It is noteworthy that the majority of researchers in this area consider that Mn oxidation takes place on the $S_2$–$S_3$ transition. It would seem intrinsically neater, more obvious and more satisfying to have a four-electron reaction carried out by four Mn, after four Mn oxidations. Indeed, the simplicity of such a picture is appealing. The ambiguities in the interpretation of the 'no Mn oxidation in $S_3$' data, described above, allow the majority view to be tenable even if it is supported by less experimental evidence. Whichever is the case, the unexpected spectroscopic properties and reactivity of $S_3$ may indicate that something interesting is happening and further study of this state is probably worthwhile. Should Mn oxidation really take place on this step, then we can conclude that water oxidation only occurs on $S_4$. If not, then there is an ambiguity: one option is that a non-substrate ligand, say, an amino acid radical is oxidized (i.e. no water oxidation); the other option is that water (as a μ-oxo bridge in the Berkeley model) is oxidized. As we shall see later, substrate-binding studies can be taken as indicating no water oxidation prior to $S_4$ formation but even this is ambiguous.

## 11. VALENCE OF THE Mn DURING THE S CYCLE

The actual valence of the Mn cluster remains debatable (for a summary, see figure 4). EPR data argue for $Mn^{III}Mn^{IV}$ (Hasegawa *et al.* 1998; Carrell *et al.* 2001; Peloquin & Britt 2001) in $S_2$ and Dismukes has argued from EPR simulations that two more $Mn^{III}$ are better candidates than $Mn^{IV}$ to make up the tetramer (Zheng & Dismukes 1996; Carrell *et al.* 2001). By contrast, the X-ray absorption K-edge data have been taken by several groups as indicating that the best candidate for $S_1$ is $Mn^{III}Mn^{III}Mn^{IV}Mn^{IV}$, (and thus $Mn^{III}Mn^{IV}Mn^{IV}Mn^{IV}$ for $S_2$) (see Dau *et al.* 2001; Robblee *et al.* 2001). Kuzek & Pace (2001) have recently reassessed these arguments and consider that (some of) the X-ray data fit with the lower valence option (i.e. $Mn^{III}Mn^{III}Mn^{III}Mn^{IV}$ for $S_2$) (see also Carrel *et al.* 2001). Conversely, more recent data, including Kβ X-ray emission spectra (Robblee *et al.* 2001), seem to argue strongly for the presence of $Mn^{IV}$ in the $S_1$ state:

i.e. the high valence option (see Yachandra 2002). The suggested presence of $Mn^{II}$ in $S_0$ could be accommodated in either model ($Mn^{II}Mn^{III}Mn^{III}Mn^{III}$ or $Mn^{II}Mn^{III}Mn^{IV}Mn^{IV}$).

Over-reduced, non-physiologically occurring S states have been demonstrated to exist upon application of an array of reductants such as hydroxylamine, hydrazine or NO. The presence of what appears to be a clear $Mn^{II}Mn^{III}$ dimer state generated by reduction with NO (Goussias *et al.* 1997; Sarrou *et al.* 1998), was recently demonstrated to be an $S_{-2}$ state (Schansker *et al.* 2002). Determination of the redox state of the two other Mn ions would resolve the valence debate.

Knowledge of the valence of the Mn is important, particularly for reaction mechanisms. The electronic characteristics of the Mn ions at different valence states greatly affects their reactivity and may be crucial in determining the nature of the chemistry taking place. Some recent models have focused on the formation of the O–O bond (in $S_4$) by the nucleophilic attack from a Ca-bound hydroxo on a $Mn^{V}$=O (Pecoraro *et al.* 1998; Vrettos *et al.* 2001). A more detailed knowledge of the valence of the Mn in the states will determine if such specific models could be valid.

## 12. DEPROTONATION EVENTS DURING THE CYCLE

The expected release of protons the enzyme during the enzyme cycle originates from three sources. (i) Four protons are generated for every $O_2$ released from the two water molecules oxidized. Deprotonation may occur prior to, or simultaneously with, electron removal from water. (ii) Proton release could occur as a result of charge compensation in or near the catalytic site with protons being relayed to the outside through hydrogen-bonded chains. The deprotonated groups would then be reprotonated by the protons coming from water when it undergoes oxidation. (iii) Proton release could occur from protein residues (at the protein–water interface) relatively far from the catalytic site under the electrostatic influence of charges accumulated at the catalytic site. Given the diverse possible origins of protons, it can be seen that it might be difficult to deduce the origins of the protons that are detected during the cycle. However, because of the effects of charges on the thermodynamics of the reactions in the active site, it seems that an understanding the enzyme mechanism will require detailed knowledge of how the protein juggles the proton chemistry during the enzyme cycle.

The protons are released in a pattern that follows the enzyme cycle. In general, what is seen is something close to a 1, 0, 1, 2 pattern for the $S_0$ to $S_1$ to $S_2$ to $S_3$ to $S_0$ steps. However, the proton release pattern is modulated by pH (Rappaport & Lavergne 1991), changing to a 1, 0.8, 1, 1.2 pattern at lower pH (Schlodder & Witt 1999). The change in the pattern could be due to a p$K$ (or p$K$s) in this pH range on the ionizable group(s) close to the surface. These groups could either be at the end of a hydrogen-bonding chain(s) (i.e. reflecting protons originating in the catalytic site) or amino acids that are influenced at a distance by charges located at or close to the catalytic site.

The 1, 0, 1, 2 pattern can be compared with data monitoring electrostatically induced band shifts of adjacent pigment molecules in the photochemical part of the reaction centre. What is observed is that on the $S_1$–$S_2$ step, a band shift occurs which is taken as indicating the presence of a relatively uncompensated positive charge appearing in the enzyme. Furthermore, there is a change in the kinetics of electron transfer after this step. The rate of formation of the TyrZ radical (through oxidation by photo-oxidized chlorophyll) is reduced from 20–40 to 50–250 ns and the reduction of the tyrosyl radical is slowed from tens to hundreds of microseconds (reviewed in Diner & Babcock 1996). The effect on the kinetics, the electrostatic effect on the pigment and the lack of proton release all contribute to a moderately convincing case that a charge is accumulated on this step (see Tommos & Babcock (2000) for an alternative explanation of the band shifts).

The electrochromic effect on the formation of $S_2$ did not change with pH (despite the fact that at low pH, 0.8 protons are released). This indicates that the pH-dependent change in the deprotonation pattern was due to the titration of a distant amino acid, which deprotonated under the electrostatic influence of the catalytic site. Therefore, the proton release at low pH on $S_2$ formation is not due to a proton coming from the catalytic site (Schlodder & Witt 1999; Rappaport & Lavergne 2001; again, however, see Tommos & Babcock (2000) for an alternative view).

The deprotonation occurring on $S_0$–$S_1$ and $S_2$–$S_3$ could reflect charge compensation at the site of formation (a proton relay to the outside) and this could involve deprotonation of the substrate water. The alternative is that these represent protons released during water oxidation, but since there is no direct evidence for water oxidation prior to the final step, this explanation is not favoured. By contrast, the two protons released on the $S_3$–$S_0$ transitions are attributed to those arising from water oxidation.

In some preparations of the enzyme, the proteins are perturbed and changes in the pattern are seen resulting in a 1, 1, 1, 1 pattern. This is taken by some as indicating that the enzyme remains charge neutral on all steps (Hoganson & Babcock 1997). Alternatively, it could reflect a perturbation of the protein, changing the p$K$ of an ionizable group at its surface, so that the electrostatic effect of the charge accumulated in the catalytic site is now able to trigger a distant protonation at a wider range of pH values (Schlodder & Witt 1999; Rappaport & Lavergne 2001). The latter seems the most probable situation and, in principle, it can be verified by studying the electrochromic changes in material that exhibits the 1, 1, 1, 1 pattern.

## 13. SUBSTRATE BINDING

A difficulty with the enzymology of this enzyme is that the solvent is also the substrate. This means that obvious experiments manipulating substrate concentrations are essentially excluded. Some experiments on substrate binding have been carried out using isotopically labelled water ($H_2{}^{18}O$) and measuring the release of labelled $O_2$ using mass spectroscopy. A system for rapid mixing and injection of substrate before and after flash illumination has allowed very useful information to be obtained. By meas-

uring the appearance of $^{34}O_2$ (i.e.$^{16}O$–$^{18}O$) and $^{36}O_2$ ( i.e. $^{18}O$–$^{18}O$), exchange rates for the two different water molecules in two distinct binding environments were demonstrated. Exchange rates for one of the waters were measured on $S_0$, $S_1$, $S_2$ and $S_3$ while for the second water site it was found to be measurable for the $S_3$ state and, recently, for the $S_2$ state. Since the two sites gave distinguishable exchange rates in $S_3$, it was concluded that the O–O bond was not formed up to that point and therefore that it must be formed upon the $S_3$–$S_4$–$S_0$ transition (see Hillier & Wydrzynski 2001; see, however, Messinger 2000). These studies represent the clearest evidence for this conclusion. Any ambiguities in this conclusion rest on whether the proposed partially oxidized intermediates (peroxide etc.) can exchange with bulk water (see Messinger 2000; Renger 2001).

It is not clear that the binding sites for the water are actually on the Mn, although this is of course expected to be the case for at least one of the substrate waters. The S-state-dependent rates of exchange of substrate water were considered to be too small to be interpreted as being due to changes in the valence of the Mn (Hillier & Wydrzynski 2001; but see Messinger 2000). Hillier & Wydrzynski (2001) concluded that the two substrate waters have two chemically distinct sites and, should these consist of Mn ions, it is unlikely that they undergo valence changes. It is of course possible that the exchangeable site does not represent binding to the Mn. The $Ca^{2+}$ or possibly a protein site, spring to mind; discussions on whether this putative non-Mn site is a catalytic one or a transit for the substrate before it shifts to the catalytic Mn site on $S_4$ have yet to benefit from pertinent experimental data. It has been speculated that the catalytic site may be protected from the substrate until $S_4$ in order to prevent premature and/or partial water oxidation (Rutherford 1989).

## 14. $CA^{2+}$ AND $CL^-$

There is one $Ca^{2+}$ required for water oxidation to take place (Debus 1992; Adelroth *et al.* 1995). A specific binding site appears during the assembly of the Mn cluster (see Ananyev *et al.* 2001) and its replacement with $Sr^{2+}$ perturbs the Mn EPR (Boussac & Rutherford 1988*b*). When the $Ca^{2+}$ is removed from its site, Mn oxidation can still take place, thus allowing the formation of $S_2$, but on the following step the normal $S_3$ state is not formed (Boussac *et al.* 1989). Instead, an abnormally stable form of the $TyrZ^{\cdot}$ appears to be formed (Gilchrist *et al.* 1995) and this interacts magnetically with the Mn (still in the $S_2$ state) (Boussac *et al.* 1989). In addition, the rate of oxidation of TyrZ is decreased by several orders of magnitude when $Ca^{2+}$ is absent (Boussac *et al.* 1992; see also Voelker *et al.* 1987; Haumann & Junge 1999). A role for $Ca^{2+}$ in the deprotonation of TyrZ seems probable. Other suggested roles for $Ca^{2+}$ include (i) controlling substrate and $Cl^-$ access to the active site (Rutherford 1989) and (ii) acting as substrate water site (Pecoraro *et al.* 1998; Vrettos *et al.* 2001); but these suggestions await support from hard evidence. The initial suggestion that Ca is close to Mn or even within its coordination sphere has been difficult to prove although recent EXAFS data argue strongly in favour (Robblee *et al.* 2001). A more detailed crystal structure should resolve the ambiguity.

$Cl^-$ is also required for oxygen evolution, although it appears that it is not an absolute requirement (Lindberg & Andréasson 1996). Removal of $Cl^-$ inhibits oxygen evolution and perturbs the Mn cluster to a variable extent depending on the precise Cl-depletion method used. The removal of $Cl^-$ by incubation in Cl-free buffer slightly perturbs the Mn, as manifest by an increase in the high spin state on the Mn cluster (the $g = 4$ EPR signal) but the enzyme continues to function at a reduced rate (Lindberg & Andréasson 1996). Lindberg & Andréasson argued that the harsher $Cl^-$ depletion methods that reversibly block enzyme function result in modifications to the enzyme that are caused by the treatments themselves and are not directly related to intrinsic $Cl^-$. When $Cl^-$ is removed by high pH shock, the high spin state is formed and the S-state cycle is blocked after $S_2$ formation in the majority of centres, while treatment with $SO_4^{2-}$ or $F^-$ inhibits the enzyme but allows radical ($TyrZ^{\cdot}$?) formation in the presence of $S_2$ at least in a fraction of the centres, giving a characteristic EPR split signal that may represent a modified version of that seen in $Ca^{2+}$-depleted PSII (see Ono *et al.* 1986*a*; Baumgarten *et al.* 1990; Boussac *et al.* 1992; van Vliet & Rutherford 1996). The electron donation rate from TyrZ to $P_{680}$ is not greatly affected on the first two flashes when $Cl^-$ is depeleted (in the $SO_4^{2-}$-treated enzyme) (see Ono *et al.* 1986*b*; Boussac *et al.* 1992) and it has been shown that $Cl^-$ is required (after $SO_4^{2-}$ treatment) to progress through specific S-state transitions (Wincencjusz *et al.* 1999).

However, none of this provides information specific enough to define the role of $Cl^-$ in the enzyme and the speculations on its role (perhaps as a direct ligand to the Mn, having a redox tuning role or regulating substrate binding in some way) do not owe much to the experimental observations. It seems possible that an improved crystal structure will provide insights that will help make sense of the extensive collection of experimental observations concerning the roles of $Ca^{2+}$ and $Cl^-$, or at least point to new avenues of research that will eventually lead to an understanding of the role of these ions in the mechanism of oxygen evolution.

## 15. MECHANISMS

There are literally dozens of theories for specific mechanisms for the oxidation of water. As we have seen, there are very few pieces of information that contribute directly to a specific model. There are many lines of evidence or arguments that weigh slightly against certain kinds of mechanisms, or marginally favour others, but the current situation is vague. We shall therefore limit ourselves to one or two representative mechanisms and attempt to highlight points of interest and some of their strengths and weaknesses.

## 16. METAL CATALYSIS

The demonstration of Mn at the heart of the enzyme has led to the assumption that Mn redox chemistry is involved and that water chemistry takes place while it is bound to Mn ions. With the recognition that a polynuclear Mn complex exists, complex schemes have been imagined involving a wide range of structures. Frequently, the struc-

tures proposed have been based on structural precedence in Mn coordination chemistry. Since the number of structures has increased, from only one or two when the $S_2$ multiline EPR signal was discovered to dozens of motifs at the present time, the scope for speculation has increased accordingly. The structural studies of the enzyme described above have managed to keep certain limits on such speculation, but these limits are fuzzy and the scope for conjecture remains great. Minor advances in structural information almost systematically trigger new mechanistic models that are both detailed and speculative. There are many proposals based around Mn-centred redox chemistry but we mention just a few examples.

### (a) *Cubane to adamantane or butterfly models*

In the textbooks from the 1980s one finds the $Mn_4$ cubane–adamantane model (Brudvig & Crabtree 1986). The key element to this model was the attractive structural chemistry in which a rearrangement to an adamantane structure occurred on a high S state associated with the formation of an O–O bond between what were bridging oxos within the Mn cluster. The weakness of this model was that, while the evidence from EPR at that time indicated, not unequivocally, a tetrameric system, there was no real experimental evidence for a cubane structure. Nor was there evidence for an adamantane structure because no spectroscopic information existed on the higher S states. X-ray absorption spectroscopy eventually ruled out symmetrical cubane and adamantane structures in those S states studied (Yachandra *et al.* 1996) and, furthermore, model adamantane structures were found to be poorly reactive. A cubane related to the butterfly (open cubane) model proved less popular but featured similar chemistry (Vincent & Christou 1987). Such chemistry is thought to occur in a synthetic cubane system upon UV-induced release of an $O_2$ molecule (Carrell *et al.* 2001). As described above, recent EPR studies have reconsidered distorted cubane structures and such structures appear to be consistent with the Mn geometry in the recently elucidated crystallographic model.

### (b) *Bridging oxo-radical*

Based on the dimer-of-dimer models from EXAFS and the lack of an X-ray absorption edge change on the $S_2$–$S_3$ transition (see above), it was proposed that a bridging oxo group underwent oxidation on this step. Inspired by a situation invoked in the chemistry of copper complexes, it was suggested that the subsequent oxidation step ($S_3$–$S_4$) involved oxidation of the adjacent bridging oxo group and that this was followed by the formation of the O–O bond (Yachandra *et al.* 1996; Robblee *et al.* 2001). The weaknesses of this model include the ambiguity in the interpretation of the data on the $S_2$–$S_3$ step as described above, the lack of positive evidence for the oxidation of the μ-oxo bridge and the substrate-binding work (described above) that appears to argue against water oxidation prior to $S_4$ (see, however, Messinger 2000).

### (c) *Bridging μ-oxo groups as bases*

Dau *et al.* (2001) have put forward an uncomplicated model based on some aspects of the experimental literature and some ideas from Krishtalik (1986). The key aspect is that water oxidation is facilitated by the μ-oxo bridges acting as bases for proton removal from water. This general idea is illustrated by a specific scheme in which, upon $S_4$ formation, one of the substrate waters is bound as a terminal oxo to $Mn^V$ and the other is H-bonded to an adjacent μ-oxo bridge. The substrate waters are oxidized by the high valence Mn ion and deprotonated by the bridging oxo in a linked process, forming a peroxo-intermediate. A different bridging μ-oxo is suggested to have a similar role in deprotonating the peroxide intermediate when it undergoes oxidation prior to $O_2$ release (Dau *et al.* 2001; see also Vrettos *et al.* 2001). This makes chemical sense and there is evidence that can be taken as indicating changes in the protonation state of μ-oxo bridges in the Mn cluster upon some S-state transitions, but direct evidence for the specific features of the model is lacking.

## 17. RADICAL–METAL CATALYSIS

The first indication that the Mn was intimately associated with an amino acid radical state came in the form of an EPR signal generated by illumination of $Ca^{2+}$-depleted PSII (Boussac *et al.* 1989). This signal was attributable to an amino acid radical interacting with the Mn in the $S_2$ redox state (Boussac *et al.* 1989). This observation triggered ideas for a role for a radical in the catalytic mechanism (Pecoraro *et al.* 1994; Pecoraro & Hsieh 2000). When the radical was assigned to a tyrosyl radical (Gilchrist *et al.* 1995), specific models appeared that involved a role for the tyrosyl radical as an abstractor of hydrogen atoms from water bound to the Mn cluster. Such models provided a new way of thinking about specific mechanistic aspects (Gilchrist *et al.* 1995; Hoganson & Babcock 1997; Tommos & Babcock 2000).

The key aspect of the model was that the tyrosyl radical has a role in water oxidation. From the EPR spectrum of the TyrZ radical (at least in the Mn-depleted enzyme), it was known that it was the neutral form (i.e. tyrosine oxidation is accompanied by deprotonation). It had been assumed in the past that this occurred at a nearby base (a histidine) and that the proton was returned to the tyrosine upon its reduction by electrons originating from the Mn cluster (and ultimately water) (Debus 1992; Diner & Babcock 1996). The close proximity of the tyrosine with the Mn, led to the suggestion that the reprotonation of the reduced tyrosine occurred through a proton originating from water. Indeed, the simultaneous reduction and protonation of the tyrosine could occur as H-atom abstraction from water (Hoganson & Babcock 1997; Tommos & Babcock 2000).

In an extreme version of this model, H-atom abstraction is seen to occur on all steps of the cycle. This has the benefit of keeping the enzyme electro-neutral on all steps, something that is thermodynamically 'a good thing' although when attempting to fit the experimental data, not as good (see § 12 for the description of the data indicating the accumulation of a charge on the $S_1$–$S_2$ transition). It also resulted in the perhaps surprising idea that water is oxidized by one electron on each step of the cycle, only to be reduced again by the Mn cluster on $S_1$, $S_2$ and $S_3$. When the $S_4$ state is reached, the deprotonated substrate is oxidized by both the TyrZ radical and the pre-oxidized Mn cluster. Another difficulty with the extreme version

of this model is that real H-atom abstraction has specific distance restrictions which may not be fulfilled. The crystal structure appears to place the Mn *ca*. 7 Å from the TyrZ (Zouni *et al.* 2001). This seems too far to allow for real H-atom abstraction. All is not lost however. Linked electron and proton transfer can still occur over this distance, so the main idea (that $TyrZ^{\cdot}$ extracts both a proton and an electron from water) is not contradicted by the distance data.

A less daring version of this kind of model, and one that seems to fit better with the experimental data, is that H-atom abstraction by the tyrosyl radical may occur only on some steps, perhaps only the higher S-state transitions. This view, combined with linked protonation and electron transfer, is an attractive compromise favoured by a number of groups (e.g. Britt 1996; Vrettos *et al.* 2001).

The H-atom abstraction model involves attractive chemistry and key aspects have been shown to be feasible in terms of thermodynamics both from experiments with synthetic clusters and from calculations (reviewed in Pecoraro & Hsieh 2000). It has been adopted by the majority of recent modellers in one form or another. Its popularity does not overcome the fact that there is, as yet, no direct experimental evidence in support of it. Nevertheless, it has provided a very nice target towards which experiments and calculations can be directed, hoping to prove or disprove key features. In this way, at least, it has been worthwhile. And it might even be correct. A recent attractive model giving a detailed mechanism is that of Vrettos *et al.* (2001). This includes metal–radical chemistry on some steps, high valence Mn chemistry, inspiration from other metalloenzymes and a sprinkling of selected features, such as roles for $Ca^{2+}$ and $Cl^{-}$ in the active site, prompted by the extensive PSII literature.

## 18. MODEL SYSTEMS

Many laboratories have made multinuclear Mn-model systems that have been relevant in understanding the properties of the Mn cluster in PSII. Few, however, have shown any relevant catalytic activity as oxidants of water (see Naruta *et al.* 1994; Limburg *et al.* 1999; Carrell *et al.* 2001). One reason for this may be that the most stable systems often used ligands, which complete the coordination sphere of the Mn, thus they do not allow exchangeable terminal substrate binding. Another possible reason for the lack of reactive Mn complexes is that an important criterion in the field of inorganic chemistry is to obtain a crystal structure as a first step in characterizing a given complex. It seems probable that such a criterion would very effectively select against any interestingly reactive complexes.

Recent efforts at connecting multinuclear Mn complexes with photochemically active systems (Ru) point the way to artificial systems that may form the basis of synthetic photodriven water oxidation (Burdinski *et al.* 1999; Magnusson *et al.* 1999). Such systems could be useful in future catalytic photocells.

## 19. PERSPECTIVES

It is clear that although much information has been obtained from research into the oxygen-evolving enzyme over recent years, it cannot be said that we know how it works. Clearly, we do not. However, the questions are at least becoming more focused even if the answers are often ambiguous. The recently published crystal structure of PSII represents the threshold of a new era in PSII research. Although the poor resolution leaves much to be desired, it seems probable that in the near future a more detailed crystallographic structure of PSII will be obtained. While this is unlikely to revolutionize our understanding of the photochemical part of PSII, even a small improvement in the resolution should provide some answers to basic structural questions concerning the catalytic part of the enzyme. Long-standing structural ambiguities raised by the spectroscopic studies should be resolved. This will allow the spectroscopy to focus on short-lived states, on more detailed structural questions (electronic structures etc.) and on mechanistic questions under the dynamic conditions of enzyme function. In short, future research into understanding the enzyme mechanism will gain new momentum when the field obtains a firm structural knowledge base. In addition, specific structural targets will greatly change the research programmes for those groups that make enzyme-inspired Mn structures as spectroscopic and catalytic models.

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## GLOSSARY

EPR: electron paramagnetic resonance
EXAFS: extended X-ray absorption fine structure
PSII: photosystem II