

Biological water-oxidizing complex: a nano-sized manganese–calcium oxide in a protein environment

Mohammad Mahdi Najafpour · Atefeh Nemati Moghaddam ·
Young Nam Yang · Eva-Mari Aro · Robert Carpentier ·
Julian J. Eaton-Rye · Choon-Hwan Lee · Suleyman I. Allakhverdiev

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Abstract The resolution of Photosystem II (PS II) crystals has been improved using isolated PS II from the thermophilic cyanobacterium *Thermosynechococcus vulcanus*. The new 1.9 Å resolution data have provided detailed information on the structure of the water-oxidizing complex (Umena et al. *Nature* 473: 55–61, 2011). The atomic level structure of the manganese–calcium cluster is important for understanding the mechanism of water oxidation and to design an efficient catalyst for water oxidation in artificial photosynthetic systems. Here, we have briefly reviewed our knowledge of the structure and function of the cluster.

Keywords Artificial photosynthesis · Photosystem II · Water-oxidizing complex · Nano-sized manganese–calcium cluster · Oxygen · Water

Introduction

Photosynthesis is a process in which light energy is converted to chemical energy and used to produce organic

compounds. It is believed that more than 3 billion years ago, organisms developed the capacity to efficiently capture solar energy and use it to power the synthesis of organic molecules using photosynthesis. The photosynthetic process set into motion an unprecedented explosion in biological activity, allowing life to prosper and diversify on an enormous scale, as witnessed by the fossil record and by the extent and diversity of living organisms on our planet today. Indeed, it was the process of photosynthesis over eons of time which has provided us with the oil, gas, and coal needed to power our technologies, heat our homes and produce the wide range of chemicals and materials that support everyday life (Allakhverdiev 2011, 2012). It is also clear that three of the great challenges facing humanity in the twenty-first century are energy supply, climate change, and global food security. Although global energy demand is expected to continue to increase, the availability of low cost energy will continue to diminish. Coupled with increasing concerns about climate change due to CO₂ release from the combustion of fossil fuels, there is now an urgent need to

M. M. Najafpour · A. N. Moghaddam
Department of Chemistry, Institute for Advanced Studies in
Basic Sciences (IASBS), Zanjan, Iran

M. M. Najafpour (✉)
Institute for Advanced Studies in Basic Sciences (IASBS),
Center of Climate Change and Global Warming,
Zanjan, Iran
e-mail: mmnajafpour@iasbs.ac.ir

Y. N. Yang · C.-H. Lee
Department of Molecular Biology, Pusan National
University, Busan 609-735, South Korea

E.-M. Aro
Department of Biochemistry and Food Chemistry,
University of Turku, 20014 Turku, Finland

R. Carpentier
Groupe de Recherche en Biologie Végétale (GRBV), Université
du Québec à Trois-Rivières, C.P. 500, Trois-Rivières,
Quebec G9A 5H7, Canada

J. J. Eaton-Rye
Department of Biochemistry, University of Otago,
P.O. Box 56, Dunedin, New Zealand

S. I. Allakhverdiev
Institute of Plant Physiology, Russian Academy of Sciences,
Botanicheskaya Street 35, Moscow 127276, Russia

S. I. Allakhverdiev (✉)
Institute of Basic Biological Problems, Russian Academy
of Sciences, Pushchino, Moscow Region 142290, Russia
e-mail: suleyman.allakhverdiev@gmail.com

develop novel clean, environmentally friendly and renewable fuels that can support energy systems to replace fossil fuels. The fuel must be made from cheap and “endless” resources that are available everywhere. Taking into account the above considerations, the goal of developing biomimetic artificial photosynthetic systems is to utilize solar energy for conversion into chemical energy through a series of electron transfer events. A promising approach for the design of such systems will be to adhere to the same chemical design principles found in natural photosynthesis (Najafpour 2006; Allakhverdiev 2011, 2012; Najafpour and Allakhverdiev 2012; Najafpour 2012).

A key step in the evolution of photosynthesis occurred when cyanobacteria evolved about 2.5 billion years ago leading to the accumulation of oxygen in the atmosphere (Wydrzynski and Satoh 2005; Govindjee 2011; Allakhverdiev 2011, 2012). The presence of oxygen made the existence of heterotrophs such as humans possible (Payne et al. 2011). There are two well-known basic parts to photosynthesis: the so-called light reactions and the subsequent series of reactions that “fix” CO₂ into carbohydrate. In the light reactions, water is oxidized and reducing power (NADPH) and ATP are produced. Biological water oxidation involves three distinct steps: (i) trapping of light by chlorophyll pigments and rapid energy transfer to the reaction centre (P680) of Photosystem II (PS II), resulting in its oxidized form (P680⁺); (ii) rapid electron transfer to P680⁺ from an oxidizable protein side-chain intermediate (tyrosine 161 of the D1 PS II reaction center protein), and (iii) electron transfer to tyrosine 161 from the manganese–calcium cluster which is the catalytic complex for water oxidation. The water-oxidizing complex (WOC), also referred to as the oxygen-evolving complex (OEC), in PS II is the heart of the water-oxidizing machinery of photosynthesis (McEvoy and Brudvig 2006; Najafpour 2006; Najafpour and Allakhverdiev 2012; Najafpour et al. 2012a, b). Following the light reactions the so-called dark reactions that do not depend directly on light are activated in the Calvin–Benson cycle. This series of reactions takes CO₂ and energy from the NADPH and ATP that were produced in the light reactions to form the carbohydrates, and subsequently the lipids and proteins that are required for life (Golbeck 2006).

Today we have considerable knowledge of the working of natural photosynthesis and its photosystems, including the water oxidation reaction. However, many questions and details remain unanswered. To fully understand the photosynthetic reactions is not only a satisfying intellectual pursuit, but is also an important goal as we strive to improve agricultural yields and develop new solar technologies for the splitting of water and for generating fuels (Najafpour 2006; Allakhverdiev 2011, 2012; Najafpour and Allakhverdiev 2012; Najafpour et al. 2012a, b). In this mini review we

present a snapshot of our current view on the structure and function of the WOC in nature and in biomimetic systems.

The importance of the water-oxidizing complex

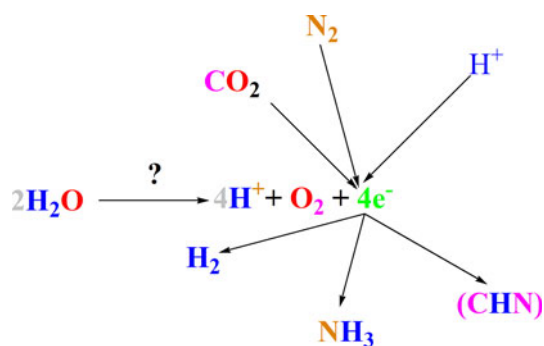
For biological water oxidation, Nature uses a manganese–calcium catalytic cluster that oxidizes water with high yield and with a turnover of up to 100–400 molecules of O₂ released per second (Dismukes et al. 2009; Duan et al. 2012). Water oxidation is not only important in natural photosynthesis but also it is important in artificial photosynthetic systems. As an example, the production of hydrogen gas, an ideal fuel for the future, from hydrogen ions (Eq. 2) via water splitting (Eqs. 1, 3) has been proposed as the holy grail of chemistry (Bard and Fox 1995). But to evolve hydrogen in a sustainable biomimetic system, it is necessary first to synthesize a “super catalyst” for water oxidation, which is the most challenging half reaction of water splitting (Bard and Fox 1995; Bockris 1977).



Water oxidation as a source for “cheap electrons” (Bockris 1977) (Eq. 1) provides electrons not only for proton reduction but also for other reduction reactions which are equally important in artificial photosynthesis (Scheme 1). Solar power is the most abundant source of renewable energy and the photosynthetic machinery provides a blue print for the use of this energy to power the thermodynamically and chemically demanding reaction of water oxidation. At the heart of the reaction is the Mn₄O₅Ca cluster of the WOC that is housed in a protein environment that controls reaction coordinates, proton movement and water access (see the latest 1.9 Å resolution structure of PS II by Umena et al. (2011)). PS II is a dimeric, multi-subunit, transmembrane protein complex of ~650 kDa molecular weight, found in the thylakoid membranes of plant and algal chloroplasts and in cyanobacteria (Najafpour and Govindjee 2011). The Mn₄O₅Ca cluster has a dimension of about ~0.5 × 0.25 × 0.25 nm. Thus, the WOC may be considered as a nano-sized manganese–calcium oxide in a protein environment.

Structure of the water-oxidizing complex

The importance of manganese in oxygenic photosynthesis was already implicit from the work of Pirson in (1937). A famous model for the WOC was the dimer-of-dimers



Scheme 1 During water oxidation electrons and protons are also produced that could be used in synthesizing fuel and useful compounds

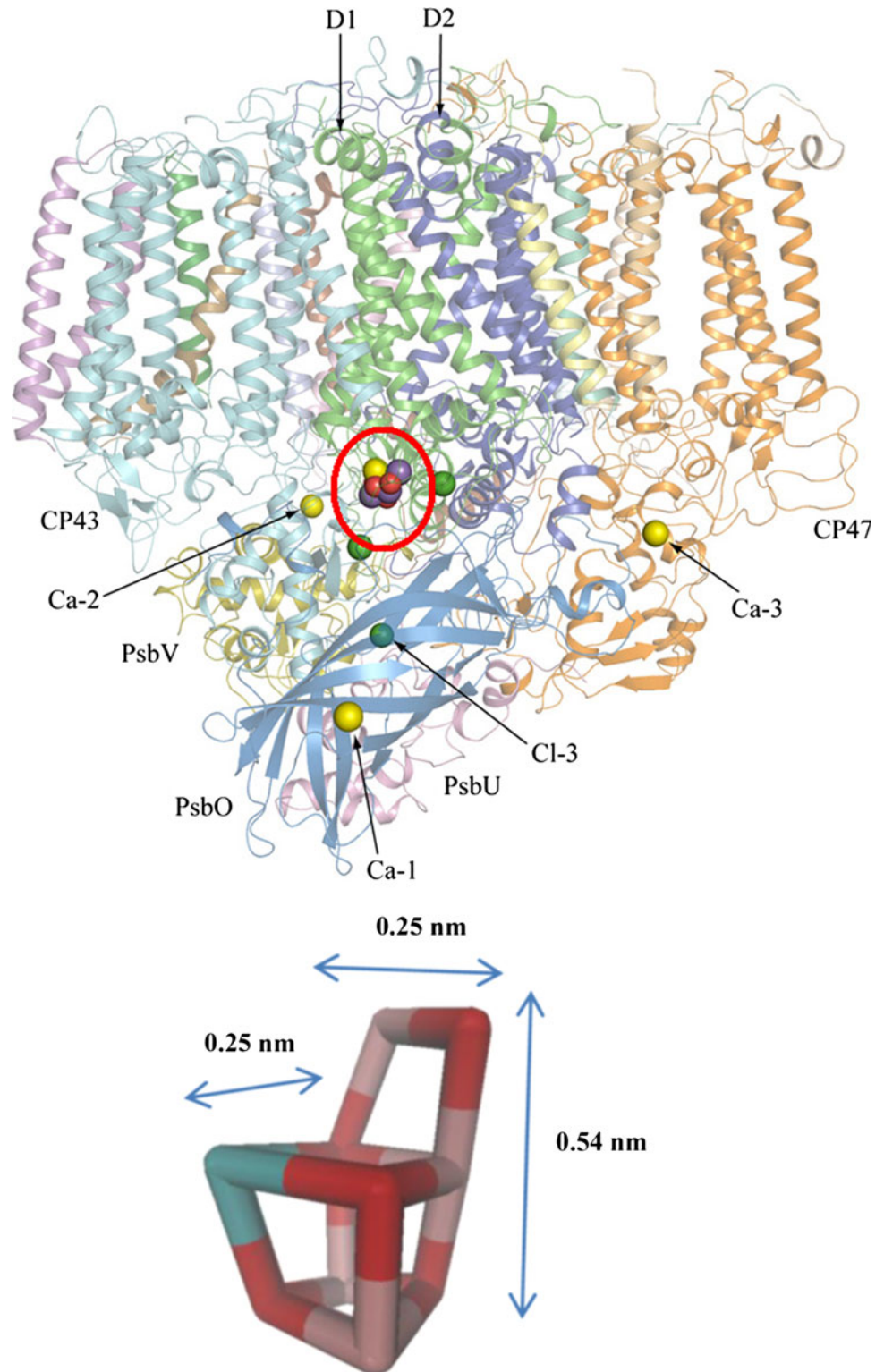
model where the Mn complex was assumed to be composed of two Mn “dimers” each being a di- μ -oxo bridged Mn_2 unit (Sauer et al. 1992). However, according to EPR data, David Britt’s group at the University of California—Davis, concluded that their electron spin echo electron nuclear double resonance experiments were difficult to reconcile with the dimer-of-dimers model and they proposed the so-called dangler model (Peloquin et al. 2000). The first papers dealing with the detailed structure of PS II were from the research groups of Horst Tobias Witt and Wolfram Saenger, in Berlin (Zouni et al. 2001) followed by the work of Nobuo Kamiya and Jian-Ren Shen in Japan (Kamiya and Shen 2003). In 2004, a detailed study was published from the research groups of James Barber and So Iwata at Imperial College, London, that provided a rather complete structure of PS II (Ferreira et al. 2004). At this point, the positioning of most of the subunits was unveiled. Importantly, for the first time, James Barber, So Iwata and colleagues provided the location of Ca in the WOC and introduced the cluster as a Mn_3Ca -cubane structure with the 4th Mn attached a bit away from the cubane core (Ferreira et al. 2004). Then, Yano et al. (2006) proposed a series of chemically related models for which the calculated EXAFS spectra matched the polarized EXAFS data obtained for PS II crystals. In other words, they performed an extended set of hypothetical models of the Mn_4Ca complex of PS II and evaluated the calculated EXAFS spectra for the three experimental spectra detected for excitation along the a, b, and c axes (Yano et al. 2006). In 2011, the research groups of Jian-Ren Shen and Nobuo Kamiya significantly improved the resolution of the PS II crystals obtained from the thermophilic cyanobacterium *Thermosynechococcus vulcanus* (*T. vulcanus*) down to atomic resolution (Umena et al. 2011; Kawakami et al. 2011). They showed that the WOC contains four manganese ions, one calcium ion, and five oxygen atoms (Umena et al. 2011; Kawakami et al. 2011) (Fig. 1). However, all X-ray studies on PS II crystals have been plagued by

X-ray-induced damage to the $\text{Mn}_4\text{O}_5\text{Ca}$ cluster, so that structures now in the literature are of something other than the native S_1 state (Yano et al. 2005) (for a detailed discussion of the S states of the WOC see Renger (2012) and the Mechanism of biological water oxidation, section below). For example, the Mn–O distances in the crystallographic model of Umena et al. (2011) are long compared with typical distances in synthetic complexes (Grundmeier and Dau 2012). Regarding the recent model, Grundmeier and Dau (2012) show that the average oxidation state of Mn in the crystal should be +2.5, corresponding on average to a Mn(II,II,III,III) system rather than a pure S_1 state with Mn(III,III,IV,IV). This observation implies that at least a fraction of the manganese ions in the enzyme are reduced.

An important issue in the new structure proposed by Umena et al. (2011) was the presence of four terminal water ligands: two of which coordinated to Ca and two to the “dangling” Mn (Mn(4)). The structure is thus a manganese–calcium cluster that can be described as a $\text{Mn}_4\text{O}_5\text{Ca}(\text{H}_2\text{O})_4$ complex. In the structure, the calcium and three manganese ions occupy four corners and four oxygen atoms form the other four corners of the cubane-like structure. It is important to note that regarding the Ca–O and Mn–O bond lengths, the cubane-like structure is not an ideal and symmetric one. In this structure, there are six ligands around Mn(1): three $\mu_3\text{-O}$ as hard ligands and two carboxylates and one imidazole group as borderline ligands that could together stabilize the oxidation number of III or IV for this manganese ion. The six ligands around Mn(2) are three $\mu_3\text{-O}$ and three bridging COO^- (Umena et al. 2011; Kawakami et al. 2011) (Fig. 2).

These ligands (Fig. 2) could stabilize the oxidation state of III or IV for this Mn(2) ion. This ion is connected to calcium and two other manganese ions with a bridging carboxylate and three oxo groups. The ligands around Mn(3) are three $\mu_3\text{-O}$, one $\mu_2\text{-O}$ and two bridging COO^- groups. Four hard $\mu\text{-O}$ ligands could stabilize manganese (IV) rather than manganese (III). The ligands around Mn(4) are one $\mu_4\text{-O}$, one $\mu_2\text{-O}$, two bridging COO^- groups and two H_2O molecules (Umena et al. 2011; Kawakami et al. 2011). These two water molecules are very important and one of them may serve as one of substrates for water oxidation (Umena et al. 2011; Kawakami et al. 2011). The ligands around Mn(4) could help to stabilize the oxidation number of (III) for this Mn ion, but de-protonation of water molecules could also participate in the stabilization of the oxidation state of (IV) as well as higher oxidation states. However, this simple analysis shows that the cluster with four or even three manganese ions with oxidation states of IV may not be stable. Analysis of various experimental, theoretical and computational results shows that higher oxidation states of the cluster in S_2 , S_3 , or S_4 with

Fig. 1 *Top* the manganese–calcium cluster (circled in red) may be considered as a nano-sized manganese–calcium oxide in a protein environment (Adapted from Kawakami et al. 2011). *Bottom* the Mn_4O_5Ca cluster in PS II that has dimensions of about $\sim 0.5 \times 0.25 \times 0.25$ nm

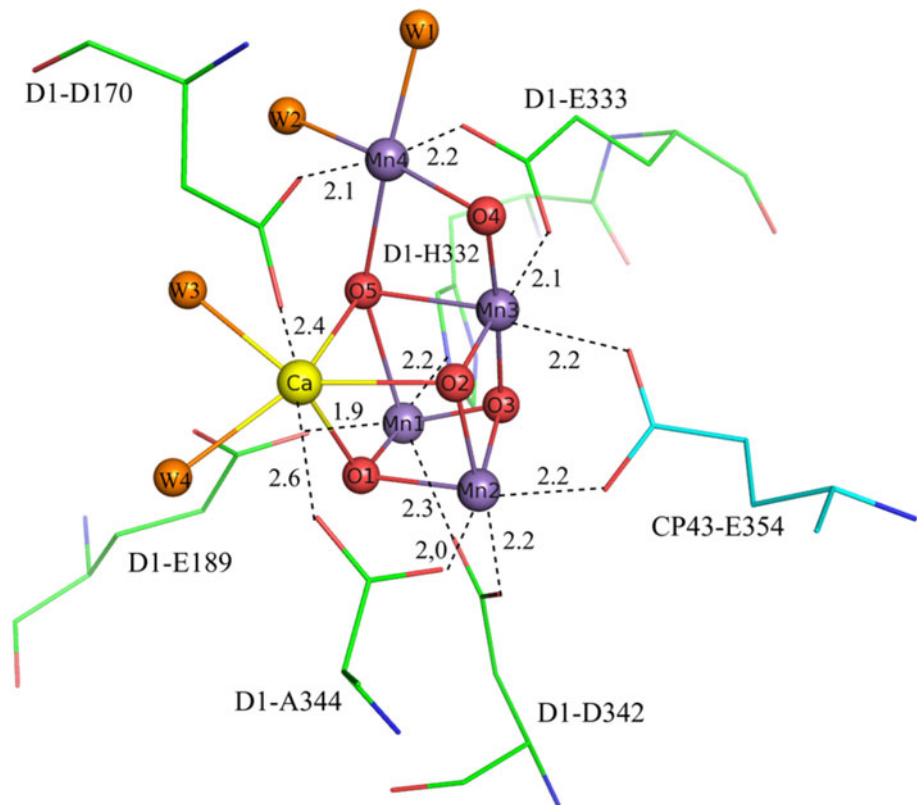


four or even three manganese ions with oxidation states of IV are unstable and decay to lower oxidation states (McEvoy and Brudvig 2006; Grundmeier and Dau 2012).

Another important ion in the WOC is calcium. The structure reported by the research team of Jian-Ren Shen

and Nobuo Kamiya shows calcium has seven ligands, three μ_3 -O bridges, two bridging COO^- groups and two H_2O molecules (Umena et al. 2011; Kawakami et al. 2011). Calcium, as a co-factor in the WOC, was found by Ghanotakis et al. (1984) and shown to be essential in oxygen

Fig. 2 Ligand structure of the metal cluster determined at 1.9 Å resolution. Residues from D1 are colored in *green*, and that from CP43 are colored in *cyan*. (Adapted from Kawakami et al. 2011)



evolution. Among many metal (II) ions, strontium (II) is the only cation that can functionally substitute for calcium in the WOC (see Boussac et al. 2004). Lee et al. (2007) provided direct support for the proposal that calcium plays a structural role in the early S-state transitions. They found the role can also be fulfilled by other cations with similar ionic radius. However, Lohmiller et al. (2012) using X- and Q-band EPR, and ^{55}Mn electron nuclear double resonance (ENDOR), showed that upon Ca^{2+} removal from the WOC, its electronic structure remains essentially unaltered. It was also proposed that Ca^{2+} could be important for the proton-coupled electron transfer between Y_z and the Mn cluster or, alternatively, may act as an initial binding site for substrate water (Lohmiller et al. 2012).

The imidazole nitrogen of D1-His 337 is hydrogen-bonded to the μ_3 -oxo bridge connecting Mn(1), Mn(2), and Mn(3). Petrie et al. (2012) proposed that such interactions may account for the lengthening of the Mn–Mn distances observed in the most recent (1.9 Å) crystal structure of PS II compared to earlier, lower-resolution (2.9 Å or greater) X-ray-derived structures and EXAFS studies on functional PS II. They used density functional theory (DFT) calculations to test the influence on Mn–Mn distances on H-bonding interactions mediated by the proximate His 337 residue and their calculations showed that the H-bonding interaction of $\text{Mn}_4\text{O}_5\text{Ca}$ with the His 337 residue leads to expansion of the “close” Mn–Mn distances (Petrie et al. 2012). The role of this hydrogen bond may be

as a stabilizer for the WOC (Umena et al. 2011; Kawakami et al. 2011) (Fig. 3).

Arginine 357 in the second coordination sphere of the WOC is an important residue that may play an important role in maintaining the structure of the metal cluster, either in stabilizing the cubane structure or by providing partial positive charges to compensate for the negative charges induced by the oxo bridges and carboxylate ligands of the WOC. One of nitrogens of CP43-Arg 357 is hydrogen-bonded to two μ -O ligands of the manganese–calcium cluster, whereas the other is hydrogen-bonded to the carboxylate oxygen of D1-Asp 170 and to that of D1-Ala 344 (see Fig. 3). The structure shows that the distances between the nitrogen atoms of the arginine side chain and Ca^{2+} are 4.2 and 4.4 Å, respectively. Also, the distances between the nitrogen atoms of the arginine side chain and Mn(4) are 4.7 and 6.0 Å, respectively. The side chain of arginine may stabilize the structure of the WOC as it is hydrogen-bonded to two μ -O bridges and one carboxylate group bridging between Ca^{2+} and Mn(2).

Two chloride ions (Cl_1 and Cl_2) are in the recent structure of the WOC (Fig. 4). Both chloride ions are surrounded by water molecules and amino acids. Calculations combined with molecular dynamics proposed that chloride depletion forms a salt-bridge between D1-Asp 61 and D2-Lys 317, the two acido-basic side chains that contribute to the high-affinity binding site for chloride. This salt bridge would modify the pK_a of D1-Asp 61 and

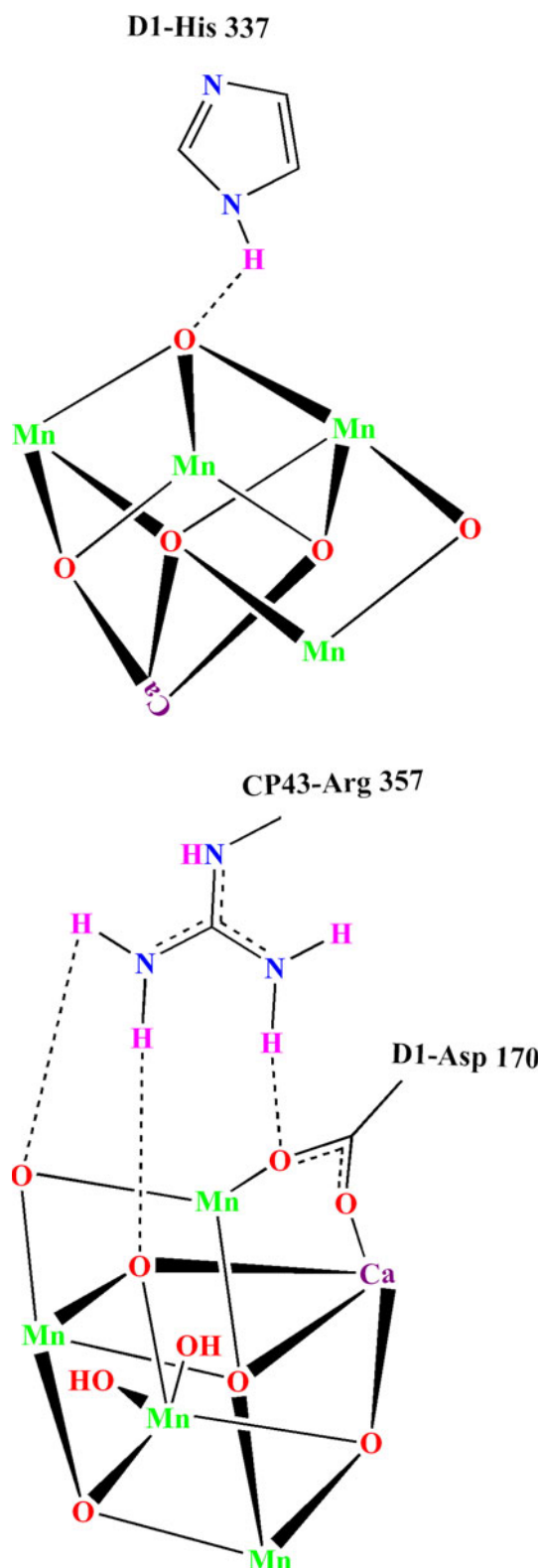


Fig. 3 *Top* the localization of D1-His 337 that is hydrogen-bonded to one of the μ -O in the WOC. *Bottom* the localization of Arg 357 (Adapted from Umena et al. 2011 and Kawakami et al. 2011)

would prevent the efficient proton release associated with the accumulation of oxidizing power within the catalytic center. The Cl₁ site would thus set the conformation appropriate for the efficient conduction of protons (Rivalta et al. 2011).

After photons are captured and excitation energy is transferred to the reaction center chlorophyll P₆₈₀, a primary charge separation occurs: oxidized P₆₈₀ (P₆₈₀⁺) and reduced pheophytin (Phe⁻) are formed (Wydrzynski and Satoh 2005). Then, P₆₈₀⁺ is reduced by electron transfer from the tyrosine (Tyrosine 161) residue (Y_z) to form a tyrosine radical ((Y_z)[•]) (Hammarstrom and Styring 2011). A hydrogen bond is observed between Y_z and the ϵ -nitrogen of a histidine (D1-His 190) (Fig. 5). The D1-His 190 was further hydrogen-bonded to other amino acids or water molecules to form a hydrogen-bond network suggested as an exit channel for protons (Umena et al. 2011; Kawakami et al. 2011). Y_z is connected to a water molecule at a distance of 2.46 Å, which in turn is connected to another water molecule that is bound to the Ca²⁺ of the WOC. Recently, Hammarstrom and Styring (2011) reviewed the possible roles of Y_z in the WOC of PS II. Firstly, Y_z is a rapid electron donor to P₆₈₀⁺. Secondly, the presence of Y_z allows the WOC to be positioned at an appropriate distance from P₆₈₀; it is argued that a closer proximity of the WOC and P₆₈₀ could lead to non-productive quenching of excited P₆₈₀ by reverse electron transfer (Hammarstrom and Styring 2011; Hammarstrom et al. 2001). Thirdly, Y_z may possibly be directly involved in the water oxidation chemistry. The proton may transfer back and forth along the hydrogen bond between Y_z and the neighbouring D1-His 190. Proton transfer to D1-His 190 may possibly control electrostatics such that oxidation of Y_z triggers deprotonation of the WOC of PS II (Hammarstrom and Styring 2011).

Three different channels for oxygen, water and protons could be found in PS II (Barber 2008; Barber and Murray 2008; Ho 2008; Ho and Styring 2008; Guskov et al. 2009). Electrostatic, structural, and orientational grounds were proposed as functional assignments of these channels. Investigations of water movement in PS II introduced a novel perspective to the study of the supply of water to the WOC and showed that functional PS II is characterized by a branched water supply structure with multiple control points.

Manganese-stabilizing protein

Nowadays it is well known that higher plants, algae and cyanobacteria all perform oxygenic photosynthesis, and the

Fig. 4 A hydrogen-bond network connecting the Mn_4CaO_5 -cluster and chloride ions (Adapted from Kawakami et al. 2011)

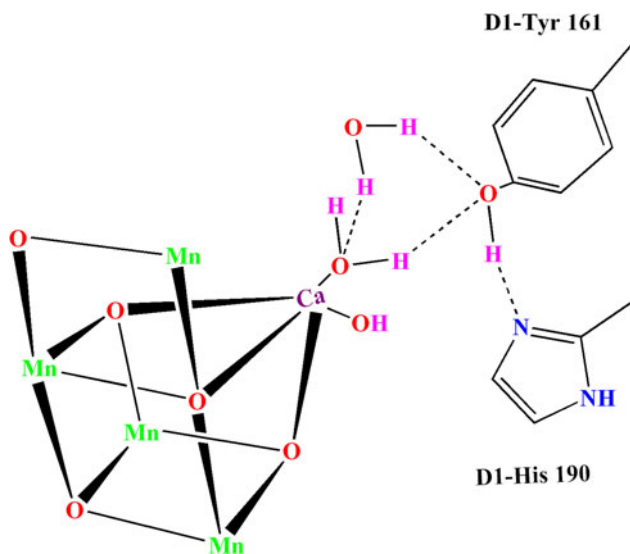
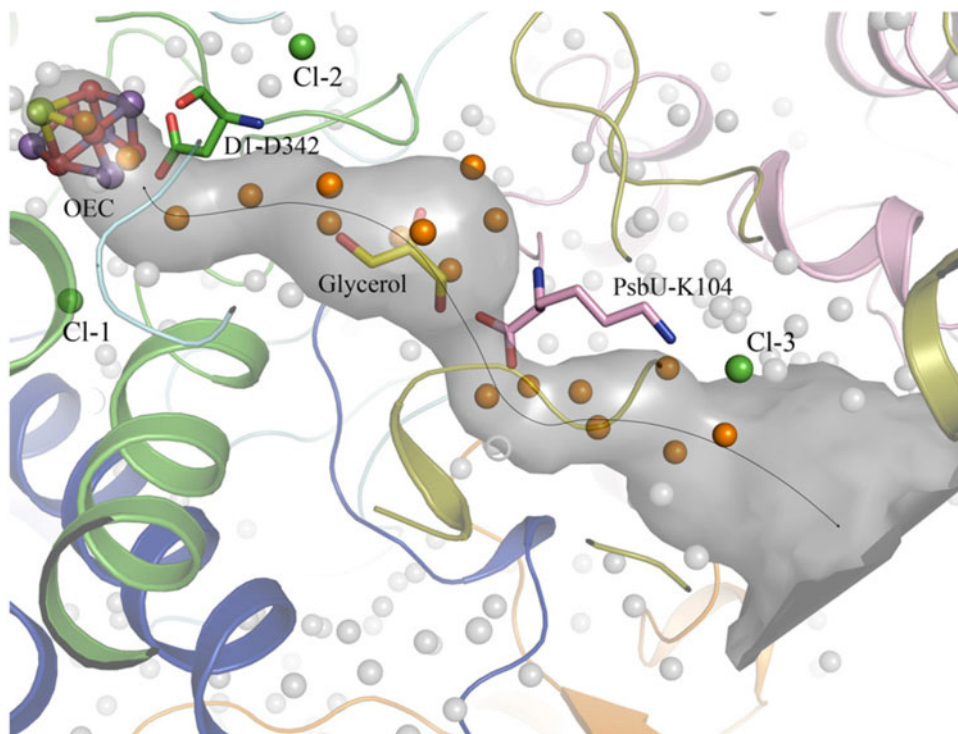


Fig. 5 There is a hydrogen bond between Y_z and the ϵ -nitrogen of the D1-His 190 that is important in proton coupled electron transfer

basic structure of their photosynthetic machinery is highly conserved. The PS II complex is composed of the core proteins, D1 and D2, which bind all the redox-active components involved in electron transfer through PS II. In addition to the D1 and D2 proteins, PS II contains the inner chlorophyll-binding antenna proteins CP43 and CP47, and the Cyt b_{559} proteins PsbE and PsbF. Moreover, several low-molecular-mass proteins are required for proper function and assembly of the PS II dimer (Komenda et al.

2012). Although the ultimate function of the WOC of PS II is very similar in the eukaryotic organisms and the prokaryotic cyanobacteria, the detailed characteristics of the individual WOC proteins differ between these organisms. Another difference in PS II between the eukaryotic and prokaryotic organisms concerns the light-harvesting machinery (see for review Aro et al. 1993; Allakhverdiev and Murata 2004; Nishiyama et al. 2006; 2011; Murata et al. 2007, 2012; Mulo et al. 2012). The WOC is also shielded from the thylakoid lumen by several extrinsic polypeptides attached to the intrinsic subunits of PS II (Bricker et al. 2012).

Photosynthetic organisms growing under variable environmental conditions are often exposed to different types of stress like harmful irradiation (UV-B or high intensity visible light), heat, cold, high salt, and also infection by pathogens (viruses, bacteria) and PS II can become unstable (Aro et al. 1993; Keren et al. 1997; Allakhverdiev and Murata 2004; Jo et al. 2010; Nishiyama et al. 2006, 2011; Wang et al. 2011; Zulfugarov et al. 2011; Murata et al. 2007, 2012; Mulo et al. 2012). The largest extrinsic protein is the manganese-stabilizing protein (PsbO) that is present in all O_2 -evolving photosynthetic organisms (Miyao and Murata 1984; Popelkova et al. 2011). PsbO is essential for stabilization and retention of the Mn and Cl^- cofactors (Bricker 1992; Eaton-Rye et al. 2003; Bricker et al. 2012). The results by Popelkova et al. (2011) showed that the protein affects the ability of calcium to protect the Mn cluster against inhibition by reduction in the dark. It was

reported with *in vitro* experiments that after depletion of PsbO from PS II using CaCl_2 or urea, water oxidation is reduced to only $\sim 20\%$ of the control value (Ono and Inoue 1983; Miyao and Murata 1984; Bricker 1992; Bricker et al. 2012). Miyao and Murata (1984) showed that incubation of PsbO-depleted PS II at low Cl^- concentrations causes the loss of two of four Mn ions from the WOC, but this loss can be prevented by the addition of a high concentration of Cl^- . Similarly, several ionic species such as metal cations, ruthenium red, or polyamines deplete PsbO from the WOC and destabilize the $\text{Mn}_4\text{O}_5\text{Ca}$ cluster but this depletion can be prevented by an increased concentration of Ca^{2+} and/or Cl^- (Beauchemin et al. 2007; Boisvert et al., 2007; Gauthier and Carpentier 2008; Hamdani and Carpentier 2009). Popelkova et al. (2006) showed by introducing specific Arg mutations in PsbO that the protein is important for retention of Cl^- in the WOC. Ådelroth et al. (1995) showed removal of PsbO either eliminates the Ca^{2+} binding site in PS II or removes a barrier that prevents rapid exchange of Ca^{2+} between the WOC and the surrounding medium (for review see Bricker et al. 2012).

Mechanism of biological water oxidation

Kok cycle

In 1969, Pierre Joliot showed that flash illumination produced an oscillating pattern of oxygen evolution that has a maximum yield on every *fourth* flash (Joliot et al. 1969; Kok et al. 1970; Mar and Govindjee 1972; Joliot and Kok 1975; Joliot 2005; Govindjee et al. 2010; Grundmeier and Dau 2012). This observation was a major advance toward understanding the mechanism of water oxidation as oxidation of *two* water molecules to produce *one* oxygen molecule requires the removal of *four* electrons. Based on the pattern of oxygen evolution, it was proposed that in a cycle of water oxidation, a succession of oxidizing equivalents is stored on each separate and independent WOC and when four oxidizing equivalents have accumulated one by one, oxygen is spontaneously evolved (see Kok et al. 1970). Each oxidation state of the WOC is known as an “S-state”, with S_0 being the most reduced state and S_4 the most oxidized state in the catalytic cycle (Fig. 6) (Kok et al. 1970). The $S_4 \rightarrow S_0$ transition is light independent and in this state oxygen is evolved. All other S-state transitions are induced by the photochemical oxidation of the PS II reaction center (P_{680}) (Kok et al. 1970).

There is general acceptance that $S_0 \rightarrow S_1$ and $S_1 \rightarrow S_2$ are metal-centered oxidations but the $S_2 \rightarrow S_3$ or the $S_3 \rightarrow S_4$ transitions are still controversial as to whether a metal-centered or a ligand-centered oxidation occurs (for a

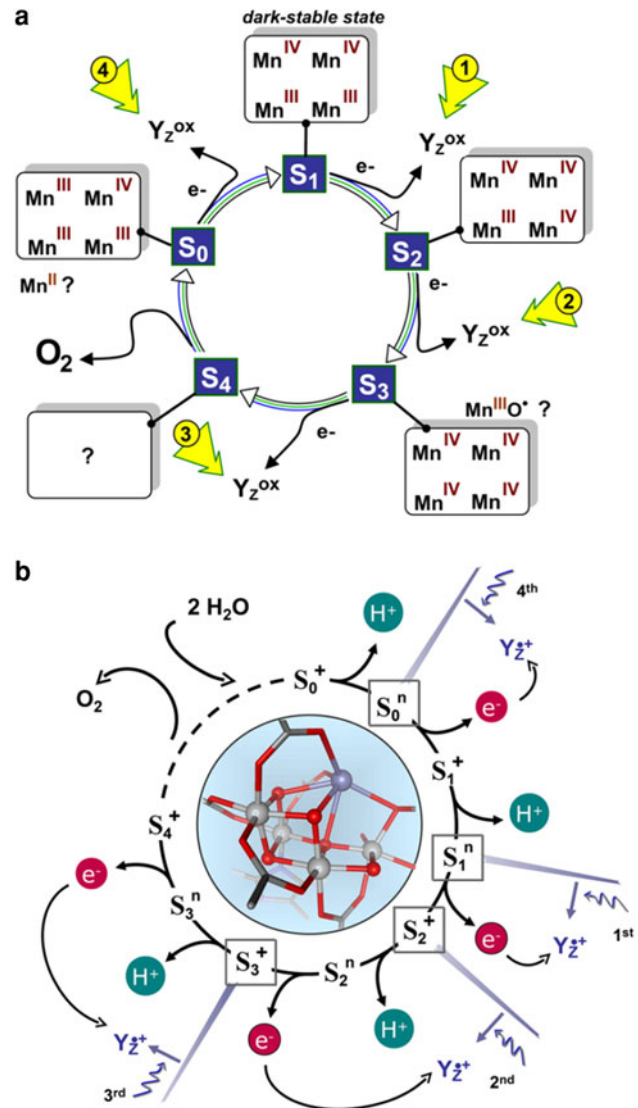


Fig. 6 Classical S-state cycle of photosynthetic water oxidation. Absorption of a photon causes charge separation at the reaction center P_{680} of PS II that leads to the formation of Y_z (oxidized tyrosine-161 on the D1-protein) within less than one μs . Reduction of Y_z by electron transfer (ET) from the manganese complex results in $S_i \rightarrow S_{i+1}$ transition; typical time constants of the ET step are indicated in the diagram. There are several similar S-state cycle schemes. Here, we show plausible oxidation-states of the four Mn ions in the different S-states (a). The extended S-state cycle including not only four oxidation but also four deprotonation steps is also shown (b) (Adapted from Grundmeier and Dau 2012)

review see Grundmeier and Dau 2012). In the $S_4 \rightarrow S_0$ transition rapid oxidation of two substrate water molecules occurs (McEvoy and Brudvig 2006). Based on the experimental evidence accumulated thus far, the four Mn ions in the S_2 -state are believed to be $\text{Mn(III)}_2\text{Mn(IV)}_2$, but a lower valence combination may also be possible. Regarding the Kok cycle, recent results show proton transfer from the WOC into the lumen occurs with a 1:0:1:2 pattern

within a strictly alternate sequence of electron and proton transfer steps (Dau and Haumann 2008). The $S_1 \rightarrow S_2$ is the only oxidation step that is not accompanied by a concomitant proton transfer.

A detailed physico-chemical mechanism of water oxidation by the WOC of PS II is still not resolved and remains a major challenge in bioinorganic chemistry. There are several proposed mechanisms for water oxidation in nature. Babcock and co-workers proposed that two different Mn ions are successively deprotonated and oxidized through hydrogen-atom abstraction by tyrosine 161 until the O–O bond is formed between the two terminally coordinated oxides or oxyl radicals (Fig. 7a) (Hoganson and Babcock 1997). However, it is apparent from the current 1.9 Å structure from *T. vulcanus* that the distance between the tyrosine 161 and manganese ions in the WOC is too large for direct abstraction of a hydrogen atom from a water species coordinated to these ions. This means that two cornerstones of the hydrogen-atom abstraction model are no longer tenable in its current form but it has inspired others to propose new models (Hammarstrom and Styring 2011; Rutherford and Boussac 2004).

The O–O bond formation (Fig. 7b) between the oxygens of the two water molecules coordinated to one manganese ion is another idea. However, Thomas Wydrzynski and colleagues in Canberra, Australia, have shown that there are two binding sites for the two substrate water molecules that can exchange their positions within the WOC only slowly (e.g., Hillier and Wydrzynski 2008). If two water-oxygens coordinated to Mn_4 were immiscible, the route of

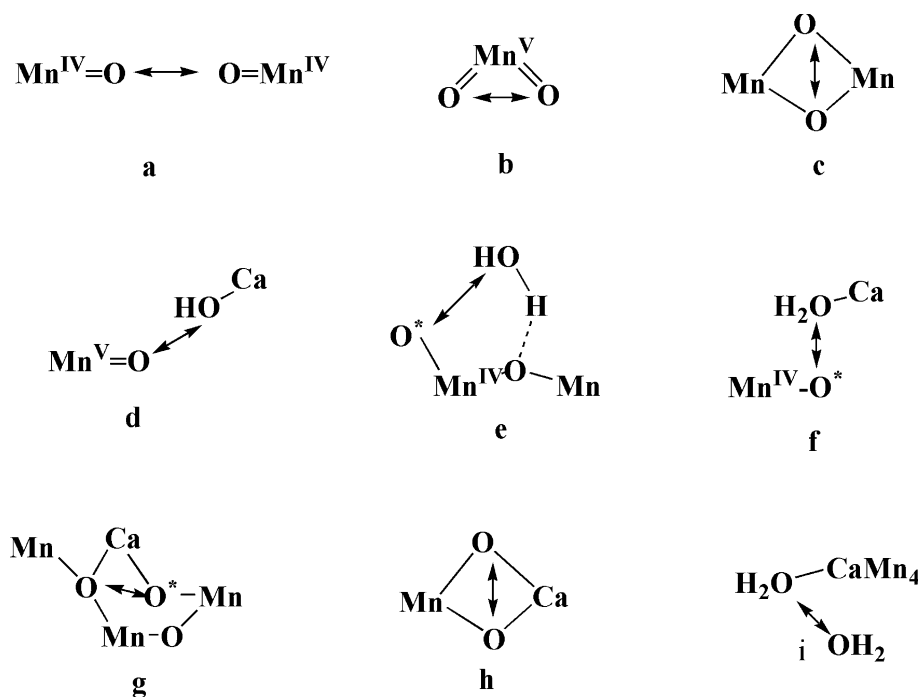
O–O bond formation shown in Fig. 7b would be in agreement with the water-exchange data.

The O–O bond formation between two bridging oxygens (Fig. 7c) has been proposed by some groups, but μ -O pairs are too inert for bond formation in the WOC. A few mechanisms for water oxidation are based on O–O bond formation by the nucleophilic attack of a water or hydroxide that either is positioned by coordination to the Ca ion or stems from outer-sphere water (Fig. 7d–f). Holgar Dau and co-workers proposed that the O–O bond could be formed by nucleophilic attack of an outer-sphere water to a $Mn=O$ that may be facilitated by the transfer of a proton from an outer-sphere substrate water to a bridging oxygen (Fig. 7e) (Dau et al. 2001). Using DFT calculations, Siegbahn (2009) has proposed that the O–O bond is formed between an oxygen radical and an oxygen atom bridged between two manganese ions and the calcium ion (Fig. 7g). Another proposed mechanism involves oxygen evolution by reaction of two bridging oxygens between one manganese ion and the calcium of the Mn_4O_5Ca cluster. It was proposed that the O–O bond could be formed by attack of an outer-sphere water to a water molecule attached to calcium that may be facilitated by Tyr-161 (Pecoraro et al. 1998; Limburg et al. 1999).

Water oxidation in artificial photosynthesis

One of the most important goals of artificial photosynthesis is to make high-energy chemicals to store sunlight (Pace

Fig. 7 Important proposed mechanisms for biological water oxidation



2005; Allakhverdiev 2011, 2012; Allakhverdiev et al. 2009, 2010; Najafpour 2011a; Najafpour and Allakhverdiev 2012). To obtain sustainable hydrogen production from water splitting, synthesizing a “super catalyst” for water oxidation is necessary (Kanan and Nocera 2008). As discussed in previous sections, there is an efficient system for water oxidation in cyanobacteria, algae and plants. To design an efficient water-oxidizing complex for artificial photosynthesis, one way could be learning and using wisely the knowledge about water oxidation and the WOC in the natural system (Hou 2010; Hou and Mauzerall 2011; Najafpour and Govindjee 2011; Najafpour and Allakhverdiev 2012; Najafpour et al. 2012a). A nano-sized compound could be defined as a particle with size in the range of 1–100 nm (10^2 – 10^7 atoms) from zero (0-D) to three dimensions (3-D). The manganese–calcium oxide has dimensions of about $\sim 0.5 \times 0.25 \times 0.25$ nm and could be considered as a nano-sized complex. Nanocompounds can exhibit unique physiochemical properties as compared with bulk ones. Two types of size effects may be distinguished with nanoscale material as compared with bulk compounds. The first effects rely on the increased surface-to-volume ratio, and the second, true-size effects which involve changes of local material properties (Jamnik and Maier 2003; Guo et al. 2002).

Nanoscale particles could have completely different redox potentials as compared with bulk material. Navrotsky et al. (2010) have reported that nanophase transition metal oxides show large thermodynamically driven shifts in oxidation–reduction equilibria. This effect could change the redox potential of nano-sized manganese and also water-oxidizing activity of nanosized manganese oxide as compared to bulk manganese oxides (Navrotsky et al. 2010). Nanosized manganese oxides are promising compounds for water oxidation because they are low-cost, environmentally friendly, and also are used by Nature to oxidize water (Harriman et al. 1987; Jiao and Frei 2010a, b; Brimblecombe et al. 2010; Hocking et al. 2011; Boppana and Jiao 2011; Najafpour 2006, 2011a, b, c; Najafpour and Govindjee 2011; Najafpour et al. 2011; Najafpour 2012; Najafpour and Allakhverdiev 2012; Najafpour et al. 2012a, b, c).

Hundreds of amino acids in PS II are also very important in water oxidation but only a small fraction of the residues, 3–4 residues on the average, are directly coordinated to the Mn_4O_5Ca cluster (Umena et al. 2011; Kawakami et al. 2011). The roles for these residues that have direct interaction with the Mn_4O_5Ca cluster could be in the regulation of charges as well as in the coordination of water molecules at appropriate metal sites, and in the stability of the cluster. The PS II manganese-stabilizing protein is a highly conserved extrinsic component of the WOC (Shutova et al. 2005). Its deletion from PS II causes a dramatic lowering of the rate of oxygen evolution (Shutova et al. 2005). The manganese-stabilizing

protein has also been suggested to be important in linking the active site of the WOC with the lumen and to be involved in a proton transfer network. A simple inorganic manganese–calcium core without coordinated amino acids shows much less activity than the WOC in PS II. Thus, the amino acids around the Mn cluster are very important indeed and the design of a *super anode* for water oxidation in artificial photosynthesis needs a deep understanding of the roles of these amino acids in water oxidation (Debus 2008). Thus, nano-sized manganese oxide attached to groups that could facilitate proton transfer, regulation of charges, and help in coordinating water molecules at appropriate sites could be an important strategy in artificial systems.

Water splitting needs not only a highly efficient water-oxidizing catalyst but also a chemically and mechanically stable catalyst. There has been much research focused on synthesizing efficient catalysts but not on their long-term stability. It is clear that biological organisms are unstable systems in an aerobic atmosphere and under environmental stresses and in such conditions PS II activity is vulnerable to inactivation. However, Nature, by employing self-repair mechanisms, has been able to solve the problem of PS II stability by extending the lifetime of sustained water oxidation (Aro et al. 1993; Keren et al. 1997; Allakhverdiev and Murata 2004; Nishiyama et al. 2006, 2011; Murata et al. 2007, 2012; Mulo et al. 2012). Incorporating a self-repair mechanism could be important in artificial model complexes. As discussed by Lutterman et al. (2009), the compounds that catalyse four-electron transfer reactions are prone to structural rearrangement, and instability, during turnover. Thus, the designs of catalysts that repair themselves are very important for energy science.

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