Quantum mechanics/molecular mechanics structural models of the oxygen-evolving complex of photosystem II

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The annual production of 260 Gtonnes of oxygen, during the process of photosynthesis, sustains life on earth. Oxygen is produced in the thylakoid membranes of green-plant chloroplasts and the internal membranes of cyanobacteria by photocatalytic water oxidation at the oxygen-evolving complex (OEC) of photosystem II (PSII). Recent breakthroughs in X-ray crystallography and advances in quantum mechanics/molecular mechanics (QM/MM) hybrid methods have enabled the construction of chemically sensible models of the OEC of PSII. The resulting computational structural models suggest the complete ligation of the catalytic center by amino acid residues, water, hydroxide and chloride, as determined from the intrinsic electronic properties of the oxomanganese core and the perturbational influence of the surrounding protein environment. These structures are found to be consistent with available mechanistic data, and are also compatible with X-ray diffraction models and extended X-ray absorption fine structure measurements. It is therefore conjectured that these OEC models are particularly relevant for the elucidation of the catalytic mechanism of water oxidation.

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Current Opinion in Structural Biology 2007, **17**:173–180

This review comes from a themed issue on
Theory and simulation
Edited by Richard Lavery and Kim A Sharp

Available online 28th March 2007

0959-440X/$ – see front matter

DOI 10.1016/j.sbi.2007.03.015

Introduction

During the past five years, there have been significant advances in the development of structural models of photosystem II (PSII). The first X-ray structure of PSII, resolving the framework of the protein complex at 3.8 Å resolution, has been reported [1]. A subsequent refinement at 3.7 Å resolution [2] enabled the assignment of the extrinsic subunits. More recently, the structure of PSII was reported at 3.5 Å resolution [3••], resolving nearly all amino acid residues and cofactors. Subsequent refinements were reported at 3.2 Å resolution [4] and 3.0 Å resolution [5••]. Although the precise positions of the individual Mn ions and substrate water molecules are yet to be resolved, the X-ray models at 3.5–3.0 Å resolution provide the overall electronic density due to the oxygen-evolving complex (OEC), and suggest possible structural models and the proteinaceous ligation scheme. However, many aspects of the X-ray diffraction structures have met with criticism, including both the geometric features of the Mn cluster [6•,7] and the proposed proteinaceous ligation [8–13]. In addition to the moderate resolution, it has been found that the X-ray diffraction data might correspond to a photoreduced Mn cluster due to the high doses of X-rays employed [14•].

Despite the incomplete and somewhat provisional nature of the X-ray diffraction structures, the proposed crystallographic models are probably the most valuable point of departure for the elucidation of complete functional models of the OEC and reaction intermediates of catalytic oxygen evolution. In fact, these recent breakthroughs in X-ray crystallography have already stimulated the development of rigorous theoretical studies [15••,16,17,18•,19•,20,21•] based on quantum mechanical analysis of reduced biomimetic model systems [16,18•,19•,20] and biomolecular modeling of the complete OEC of PSII, including the perturbational influence of the surrounding protein environment [15••,17,21•]. This review summarizes recent advances in the development of structural models of the OEC of PSII, with emphasis on quantum mechanics/molecular mechanics (QM/MM) computational structural models that include an explicit treatment of the biomolecular environment surrounding the OEC [15••,16,17,21•].

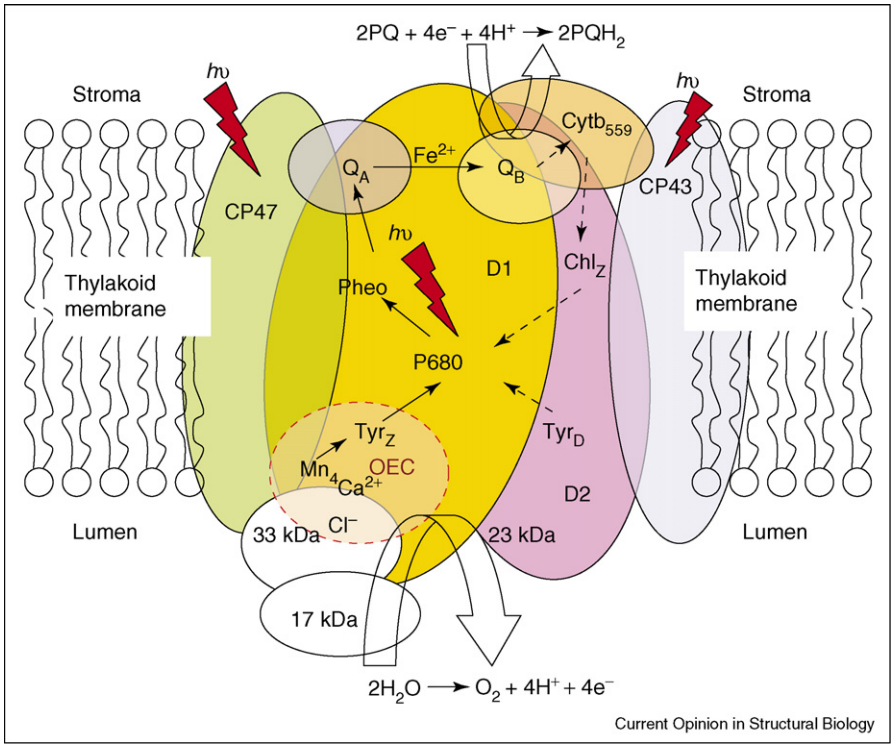
Photosynthetic oxygen evolution

PSII is a multisubunit transmembrane oxidoreductase protein complex, found in the thylakoid membranes of green-plant chloroplasts and internal membranes of cyanobacteria. The biological function of PSII is to absorb solar light and reduce plastoquinone, producing oxygen by water oxidation according to the following reaction:

$$2H_2O + 2PQ + 4H^+_{stroma} \rightarrow O_2 + 2PQH_2 + 4H^+_{lumen}$$

where PQ is a reversibly bound plastoquinone (Q_B), which forms plastohydroquinone PQH_2 after two-electron reduction and double protonation by protons

Figure 1



Overall structure of PSII. The major polypeptide subunits and ET pathway (marked by solid arrows) are shown [23–27]. Broken arrows indicate secondary ET pathways, which may play a photoprotective role.

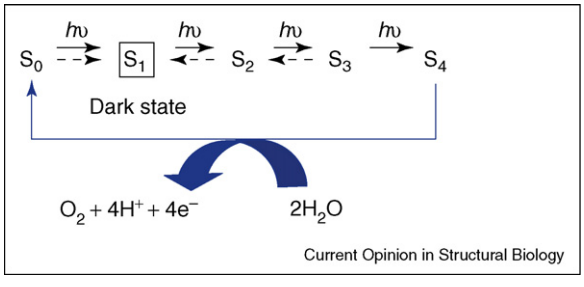
from the stromal side of the membrane [22]. PQH_2 is displaced by another PQ from the membrane pool and released as neutral dihydroquinol, as its binding affinity for the Q_B cavity is relatively low [23]. Protons from H_2O are released into the lumen, establishing the membrane pH gradient necessary for ATP synthesis. Oxygen is released to the atmosphere.

A complete description of the underlying photosynthetic light reaction remains elusive, although extensive work over many years of study has provided considerable insight into the overall reaction mechanism (see Figure 1) [23–27]. Solar energy absorbed by the antenna system (CP43 and CP47) is transferred to the specialized chlorophyll species P680, triggering a chain of electron transfer (ET) reactions across the membrane. The excited singlet state of P680 decays to the oxidized state, $P680^+$, by ET to a nearby pheophytin (Pheo) within approximately 2 ps after photoexcitation of P680. The charge-separated state is stabilized by ET to a primary quinone electron acceptor (Q_A), which functions as a one-electron carrier, and subsequently to a secondary plastoquinone electron acceptor (Q_B), which functions as a two-electron carrier and exchanges with free quinone upon two-electron reduction and protonation. The photooxidized chlorophyll species $P680^+$ is reduced by a redox-active tyrosine (Y_Z), which is, in turn, reduced by the oxidation of water catalyzed by the OEC.

Oxygen-evolving complex of photosystem II

The OEC of PSII is a high-valent manganese- and calcium-containing cofactor that catalyzes oxygen evolution by water cleavage, according to the so-called 'S-state' catalytic cycle (see Figure 2) proposed by Joliot and Kok [28,29]. Substrate water molecules are believed to ligate to metal ions in the OEC early in the catalytic cycle, as suggested by pulsed electron paramagnetic resonance (EPR) spectroscopy [30,31•], near IR Raman spectroscopy [32] and Fourier transform IR spectroscopy

Figure 2

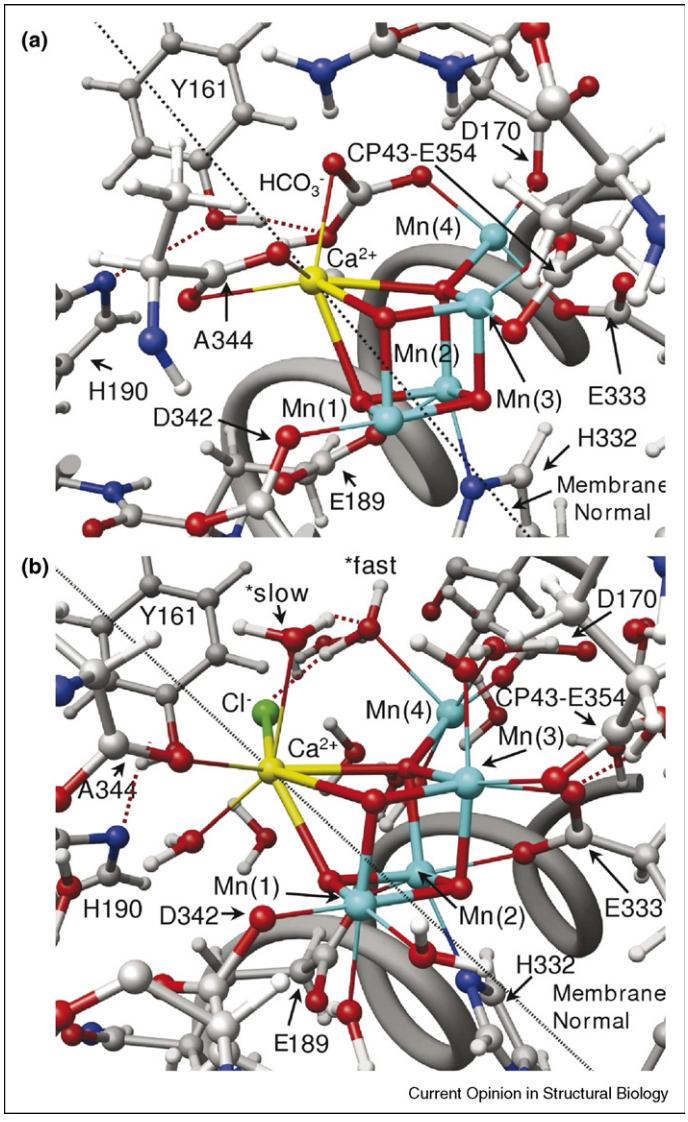


Kok catalytic cycle for photosynthetic water oxidation by reduction of the OEC of PSII, during the $S_4 \rightarrow S_0$ oxidation state transition. Dotted arrows indicate reactions that relax the system to stable state S_1 within minutes. For simplicity, deprotonation reactions during the $S_0 \rightarrow S_1$, $S_2 \rightarrow S_3$ and $S_3 \rightarrow S_4$ oxidation steps are omitted.

[33,34]. In fact, direct scrutiny based on time-resolved mass spectrometry (MS) has determined the exchange rates (k_{ex}) with bulk ^{18}O-labeled water of the two substrate waters of the OEC in the S_0, S_1, S_2 and S_3 states (reviewed in [35•]). However, neither of the two substrate water molecules has been resolved by X-ray crystallography so far (Figure 3a).

Computational structural models of the OEC of PSII have been constructed by applying state-of-the-art QM/MM hybrid methods, based on density functional theory (DFT) [15••], in conjunction with the X-ray crystal structure of PSII from the cyanobacterium *Thermosynechococcus elongatus* (PDB code 1S5L) [3••]. The QM/MM models have identified possible binding sites for the substrate water molecules (see Figure 3b), namely Mn(4) and Ca^{2+} on the 'active face' of the OEC. This arrangement is consistent with previous mechanistic proposals [17,21•,24,36]; the respective substrate oxygen atoms are 2.72 Å apart in the S_1 state and might be brought closer together after deprotonation of the Mn-bound water to achieve O–O bond formation during the $S_4 \rightarrow S_0$ transition. The substrate water molecules in the QM/MM models rationalize the electronic density in the 1S5L structure initially assigned to bicarbonate (Figure 3a). The QM/MM hybrid models also differ from the X-ray structure with regard to the nature of proteinaceous ligation and exhibit η^2-coordination of D1-E333,

Figure 3



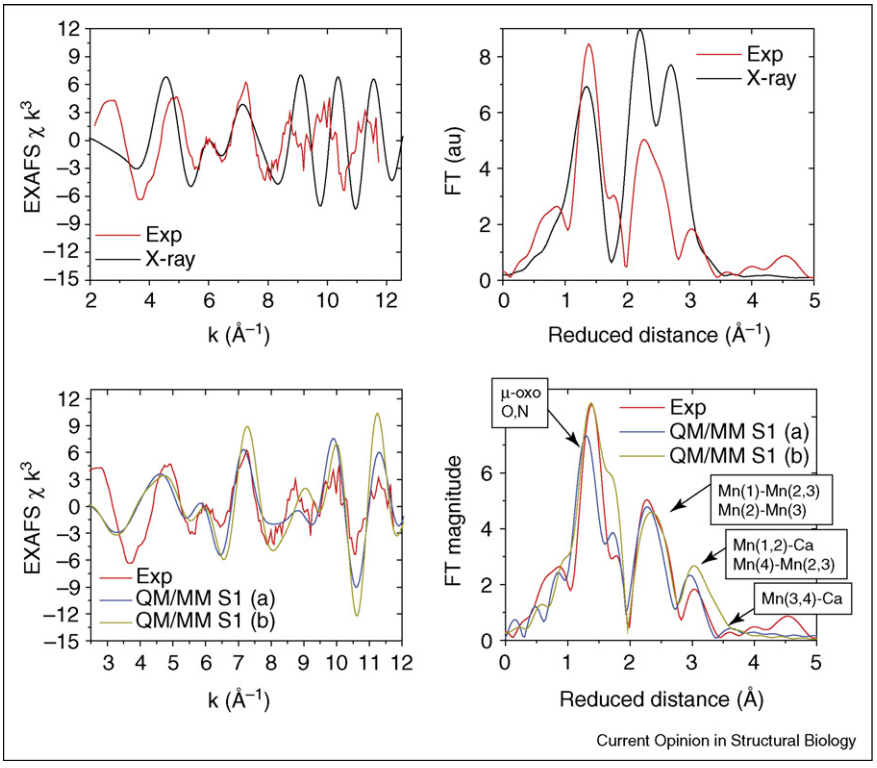
Structural models of the OEC of PSII as described by **(a)** the 1S5L X-ray crystallographic model and **(b)** DFT QM/MM hybrid models obtained at the ONIOM-EE (UHF B3LYP/lacvp,6-31G(2df),6-31G:AMBER) level. Putative substrate waters labeled *fast and *slow are coordinated to Mn(4) and Ca^{2+}, respectively. All amino acid residues correspond to the D1 protein subunit unless otherwise indicated.

where D1 indicates the protein subunit, to both Mn(3) and Mn(2), and hydrogen bonding between the carboxylate group of D1-E333 and the protonated carboxylate group of CP43-E354 in its neutral state. Also seen is monodentate coordination of D1-D342, CP43-E354 and D1-D170 to Mn(1), Mn(3) and Mn(4), respectively, and ligation of D1-E189 and D1-H332 to Mn(2). In addition, the QM/MM hybrid models include a calcium-bound chloride ion positioned 3.1 Å from Ca^{2+} and 3.2 Å from the phenoxy oxygen of D1-Y161, which is thought to be the redox-active tyrosine. Such an arrangement of ligands completes a coordination sphere of seven ligands for Ca^{2+}. The incorporation of chloride in the computational models is consistent with pulsed EPR experiments on the OEC [37] in which the chloride ion has been replaced by acetate, revealing a distance of 3.1 Å from the methyl deuterons of the bound acetate to the phenoxy oxygen of D1-Y161 [21•]. Although the presence of the chloride ion is yet to be resolved by X-ray diffraction experiments, the chloride-binding site suggested by the QM/MM models is consistent with the experimental observation that acetate binds competitively with chloride [38] and blocks catalysis at the S_2 state [39]. Such a binding site is also consistent with the proposal that chloride is part of a proton-relay network [40].

Oxygen-evolving complex oxidation states

QM/MM hybrid methods offer the most rigorous available methodologies capable of modeling the intrinsic electronic properties of prosthetic groups embedded in biological molecules (e.g. the OEC of PSII) and the influence of the surrounding protein environment. In particular, analysis of fully relaxed QM/MM configurations can provide biologically relevant insight into the structural and electronic nature of the OEC, because the effects of temperature on the PSII structure, protonation state and charge localization are negligible, as recently reported by studies of X-ray absorption at 20 K and room temperature [6•]. Several QM/MM structural models based on the 1S5L X-ray crystal structure [3••] were found to be in good agreement with the X-ray diffraction structure. The QM/MM structural models differ only in the protonation state, the number of ligated water molecules or the coordination of labile ligands. However, only two combinations of spin states were found to have comparable stability in the S_1 resting state. These combinations were found in model a, in which the dangling manganese Mn(4) is pentacoordinated and the oxidation states are Mn(1) = IV, Mn(2) = IV, Mn(3) = III, Mn(4) = III [designated Mn_4(IV,IV,III,III)]; and model

Figure 4



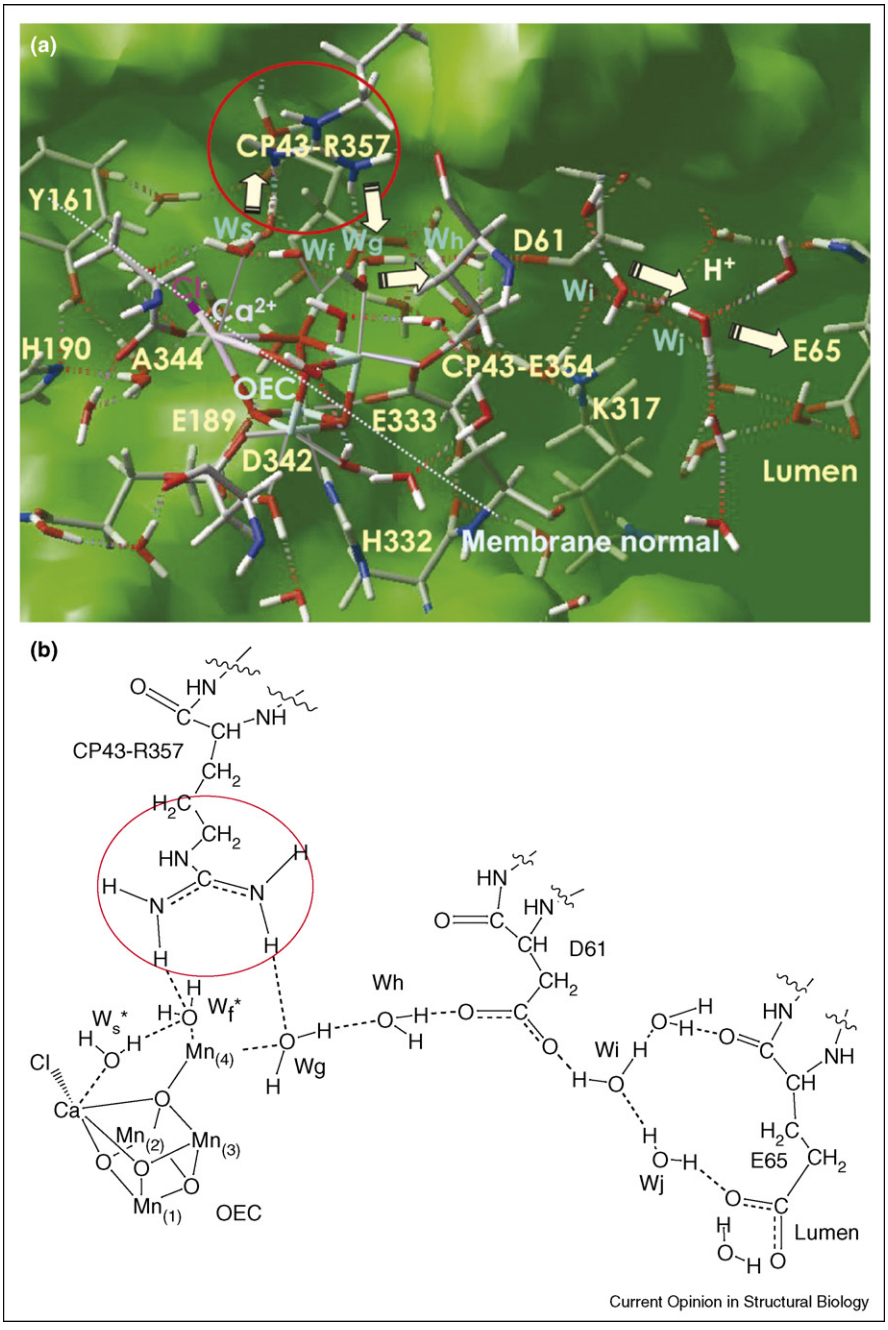
Comparison of experimental (red) and calculated (blue, green and black) EXAFS spectra in k-space (left), and Fourier transforms of the EXAFS spectra in reduced-distance space (right) for the OEC of PSII, as described by the 1S5L X-ray diffraction model (top) and the DFT QM/MM models of the S_1 state, obtained at the ONIOM-EE (UHF B3LYP/lacvp,6-31G(2df),6-31G:AMBER) level. Models include a, in which the dangling manganese is pentacoordinated and the oxidation states are Mn(1) = IV, Mn(2) = IV, Mn(3) = III, Mn(4) = III; and b, in which the dangling manganese is hexacoordinated with an additional water and the oxidation states are Mn(1) = IV, Mn(2) = III, Mn(3) = III, Mn(4) = IV.

b, in which the dangling manganese is hexacoordinated with an additional water and the oxidation states are Mn_4(IV,III,III,IV). Both models have complete coordination of the high-valent Mn centers, with oxidation states III and IV and five or six ligands, respectively. These results are consistent with EPR [41,42] and X-ray spectroscopic evidence [43,44], but disagree with low-valent Mn_4(III,III,III,III) proposals [45,46].

Oxygen-evolving complex EXAFS spectra

Recent theoretical studies have also reported the calculation of extended X-ray absorption fine structure

Figure 5



Proton exit channel leading to the lumenal surface of the membrane, as suggested by DFT QM/MM structural models: **(a)** DFT QM/MM structural model; **(b)** schematic representation. An extended network of hydrogen bonds leads from substrate water molecules Ws and Wf to CP43-R357 and from CP43-R357 to D1-D61. D61 is the first residue of the putative proton-transfer channel leading to the lumenal surface of PSII via hydrogen-bonded water molecules Wg, Wh, Wi and Wj. Note that all amino acid residues correspond to the D1 protein subunit, unless otherwise indicated.

(EXAFS) spectra, based on the real space Green's function approach as implemented in the *ab initio* self-consistent FEFF8 code (version 8.2) [47,48]. The calculations were applied in conjunction with DFT QM/MM structural models of the OEC of PSII (models a and b) [15••]. The computational task involves solving the multiscattering problem associated with the photoelectrons emitted by the Mn ions upon absorption of X-rays. The quantum mechanical interference of outgoing photoelectrons with the scattered waves from atoms surrounding the Mn ions gives rise to local maxima and minima in the oscillations of EXAFS intensities. The Fourier transform of these oscillations determines the distance to the scattering centers, including Mn–ligand and Mn–Mn distances, the coordination of Mn ions, and changes in Mn coordination with changes in the oxidation state of the metal centers. Figure 4 shows a direct comparison of calculated and experimental [6•] EXAFS spectra in k-space (experimental data were kindly provided by Holger Dau), as well as the Fourier transform of the EXAFS spectra. Calculations of the EXAFS spectrum based on the 1S5L X-ray structure are included for comparison purposes. It is shown that EXAFS spectra based on the QM/MM models are in very good agreement with experimental data, including the description of the peaks associated with multiscattering from Mn ligands at ~1.8 Å (reduced distances ~1.4–1.6 Å), the short Mn–Mn distances at ~2.7 Å (reduced distances ~2.3–2.5 Å) characteristic of PSII, and backscattering due to the dangling Mn and Ca^{2+} at ~3.3–3.4 Å. Calculations based on the X-ray structure are in much less agreement with the experimental EXAFS spectrum, mainly because of differences in the proteinaceous ligation scheme and the incomplete coordination of metal centers.

Function of D1-Y161 (Y_Z) and CP43-R357

Tyrosine D1-Y161 has long been viewed as an electron transport cofactor in PSII [49,50]. As mentioned in the introduction, the oxidized state $P680^+$ is thought to be reduced by the redox-active tyrosine (Y_Z; D1-Y161 in the case of PSII from *T. elongatus*), which is, in turn, reduced by an electron from the OEC. The QM/MM structural models seem to be consistent with such a postulated redox mechanism, especially judging by the proximity of Y_Z to the Mn cluster. Simple inspection of the QM/MM structural models (see Figures 3 and 5) also reveals that the Y_Z phenol group is hydrogen bonded to the imidazole ε-N of the D1-H190 sidechain. This hydrogen-bonding partnership is consistent with mutational and spectroscopic studies [51–53], as well as with earlier studies based on MM models [17,21•].

The possibility that the oxidized Y_Z radical might simultaneously oxidize and deprotonate the hydrated OEC [54] would require a mechanism in which Y_Z abstracts hydrogen atoms and delivers protons to the protein surface via D1-H190. However, given the lack of a hydrogen-bonding pathway leading from D1-H190 to the lumen in the QM/MM structural models, it is more likely that D1-H190 accepts a proton from Y_Z during the oxidation of Y_Z and returns the proton to Y_Z upon its reduction. Consistently, the models suggest that another amino acid residue (i.e. CP43-R357) might be more favorably placed for proton abstraction (see Figure 5).

Proton exit channel

The proposed alternative proton abstraction mechanism is also consistent with a network of hydrogen bonds formed around the catalytically active face of the OEC cluster (Figure 5), including the substrate water molecules, the sidechain of CP43-R357 and the calcium-bound chloride ion. Nearby, two hydrogen-bonded non-ligating water molecules are found to fit easily into the structure between Mn(4) and D1-D61, the first residue of the putative proton-transfer channel leading to the lumenal surface of PSII. The proximity of CP43-R357 to the Mn cluster in the QM/MM hybrid models suggests that CP43-R357 might play the role of the redox-coupled catalytic base in the latter half of the S-state cycle [55•]. A recent computational study indicates that the pK_a of D1-R357 is indeed particularly sensitive to an increase in the charge of the Mn–Ca cluster [56].

Conclusions

This article outlines recent advances in the emergence of DFT QM/MM structural models of the OEC of PSII. The reviewed computational work addresses fundamental questions that, for many years, have remained largely elusive to rigorous first-principles examination, including the architecture of the catalytic center for water oxidation, the coordination of substrate water molecules, the proteinaceous ligation scheme, the coordination of chloride, the characterization of the proton exit channel and the potential role played by amino acid residues in close contact with the catalytic inorganic core as redox-coupled basic species. The reviewed studies reveal that state-of-the-art DFT QM/MM hybrid methods constitute powerful tools for modern computational chemistry when applied in conjunction with crystal structures, even at moderate resolution. These methods are thus expected to make many more important contributions to studies of catalytic water oxidation and to the general field of chemical reactivity in biological molecules.

Acknowledgements

VSB acknowledges supercomputer time from the National Energy Research Scientific Computing (NERSC) center and financial support from Research Corporation, Research Innovation Award (# RI0702), a Petroleum Research Fund Award from the American Chemical Society (PRF # 37789-G6), a junior faculty award from the F Warren Hellman Family, the National Science Foundation (NSF) Career Program Award (CHE # 0345984), the NSF Nanoscale Exploratory Research (NER) Award (ECS # 0404191), the Alfred P Sloan Fellowship (2005–2006), a Camille Dreyfus Teacher-Scholar Award for 2005, and a Yale Junior Faculty Fellowship in the Natural Sciences (2005–2006). GWB acknowledges support from National Institutes of Health grant GM32715.

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