Energetics of proton release on the first oxidation step in the water-oxidizing enzyme

Keisuke Saito¹²³, A. William Rutherford⁴ & Hiroshi Ishikita¹²

In photosystem II (PSII), the Mn₄CaO₅ cluster catalyses the water splitting reaction. The crystal structure of PSII shows the presence of a hydrogen-bonded water molecule directly linked to O4. Here we show the detailed properties of the H-bonds associated with the Mn₄CaO₅ cluster using a quantum mechanical/molecular mechanical approach. When O4 is taken as a μ-hydroxo bridge acting as a hydrogen-bond donor to water539 (W539), the S₀ redox state best describes the unusually short O4–OW539 distance (2.5 Å) seen in the crystal structure. We find that in S₁, O4 easily releases the proton into a chain of eight strongly hydrogen-bonded water molecules. The corresponding hydrogen-bond network is absent for O5 in S₁. The present study suggests that the O4-water chain could facilitate the initial deprotonation event in PSII. This unexpected insight is likely to be of real relevance to mechanistic models for water oxidation.
The core of the photosystem II (PSII) reaction center is composed of D1/D2, a heterodimer of protein subunits that contains the cofactors involved in photochemical charge separation, quinone reduction and water oxidation. In PSII, the Mn$_4$CaO$_5$ cluster catalyses the water splitting reaction: $2\text{H}_2\text{O} \rightarrow \text{O}_2 + 4\text{H}^+ + 4\text{e}^-$ (reviewed in refs 1,2). The release of protons has been observed in response to changes in the oxidation state (the S$_0$ state, where the subscript represents the number of oxidation steps accumulated) of the oxygen-evolving complex and occurs with a typical stoichiometry of 1:0:1:2 for the S$_0$→S$_1$→S$_2$→S$_3$→S$_4$ transitions, respectively (for example, refs 3,4). Although the relevant pathways for proton transfer (PT) in each S-state transition is not yet clear, PT may proceed via different pathways in the PSII protein, depending on the S-state transition$^{5-7}$. Candidates for the relevant PT pathways have been reviewed recently$^{8-12}$ and site-directed mutagenesis studies are testing the various possibilities$^{13}$.

The majority view in the current literature is that the best resolved X-ray crystal structure (1.9Å) of the Mn$_4$CaO$_5$ cluster$^{14}$ represents an over-reduced form due to its reduction by the X-ray beam (for example, ref. 1 but see also for example, ref. 15 for an alternative explanation). This was suggested to explain the elongation of Mn–Mn and Mn–O distances compared with those obtained from extended X-ray absorption fine structure (EXAFS; for example, refs 16–21). Detailed oxidation states of the 1.9Å crystal structure are discussed in recent theoretical studies (see ref. 22 and references therein). Electron spin echo envelope modulation (ESEEM) and electron-nuclear double resonance (ENDOR) studies have suggested that all of the μ-oxo bridges of the Mn$_4$CaO$_5$ cluster are deprotonated in the S$_4$ state, and that the water molecules, W1 and W2, bound to Mn4 are H$_2$O or OH$^-$ (refs 23,24). Because proton release is not observed in the S$_1$–S$_3$ transition, the ESEEM and ENDOR data thus imply that the μ-oxo bridges of the Mn$_4$CaO$_5$ cluster are already deprotonated in S$_1$ (refs 16,19–21,25).

The 1.9Å structure revealed that two water molecules, W1 and W2, were ligands to the Mn4 atom of the Mn$_4$CaO$_5$ cluster, and two waters, W3 and W4, were ligands to the Ca atom$^{14}$. W3 has been proposed as a candidate deprotonation site because it is one of the closest water molecules to the redox active Tyr$^{12}$ and because the W3–Sr bond in the Mn$_5$S$_2$O$_5$ cluster was significantly longer compared to that in the Mn$_4$CaO$_5$ cluster$^{14}$. All of these bound water molecules are candidates not only as potential substrates for water oxidation (for example, refs 14,27–32) but also as ionizable groups that could undergo deprotonation during the enzyme cycle in which positive charge equivalents are accumulated$^{21}$.

In recent experimental (for example, ref. 33) and theoretical studies (for example, refs 34,35) it has been proposed that the proton released on the S$_3$–S$_1$ transition involves hydroxyl form of the hydroxide ion in S$_1$. This was based on the observation of a significantly long distances between O5 and the adjacent Mn ions$^{36}$. This would be conflict with the already deprotonated μ-oxo bridges in S$_1$ suggested from the ESEEM and ENDOR data$^{19-21}$. Recent report comparing published quantum mechanical/molecular mechanical (QM/MM) data and EXAFS data$^{37}$ with the free electron laser structure$^{36}$ suggested that the crystals used for this study could contain significant amount of the reduced S$_0$ state$^{38}$. Notably, the activation energy is specifically low in the S$_0$–S$_1$ transition, and can be rationalized as a rate-limiting electron transfer followed by a deprotonation step$^{37}$.

The 1.9Å structure shows the presence of a chain of strongly H-bonded eight-water molecules (O4-water chain) directly linked to O4 (linking Mn4 and Mn3 in the Mn$_5$CaO$_5$-cubane), whereas O5 has no direct H-bond partner$^{14}$. As far as we are aware, none of the QM/MM studies have considered the O4-water chain explicitly. In the 1.9Å structure, the water molecule W539 (B-factor: monomer A = 23.9, monomer B = 25.2) is situated near the O4 atom of the Mn$_4$CaO$_5$ cluster$^{14}$. Remarkably, the H-bond between O4 and W539 (O4–O$_{W539}$) is unusually short (< 2.5 Å) in comparison with typical O–O distances of ∼2.8 Å for standard (asymmetric) H-bonds in H$_2$O (refs 39,40), even if we consider the uncertainty of ∼0.16 Å in the measured distances within the 1.9Å structure$^{14}$. Notably, ‘single-well H-bonds’ are very short and typically have O–O distances of 2.4–2.5 Å (refs 41,42). This results in an essentially barrier-less potential between the H-bond donor and acceptor moieties. The appearance of single-well H-bonds in protein environments is often associated with PT events$^{43-45}$.

Here we look for short H-bonds in the environment of the Mn$_5$CaO$_5$ complex and investigate their significance in energy terms by adopting a large-scale QM/MM approach based on the crystal structure analysed at 1.9Å resolution$^{14}$.

**Results**

**A short H-bond between O4 and W539.** The O4–O$_{W539}$ bond is unusually short in the original 1.9Å structure (2.50 Å, Fig. 1)$^{14}$. When an H-bond is assumed for the O4–O$_{W539}$ bond, two H-bond patterns are possible. Case 1: the H atom is from O4, an OH$^-$ moiety, so that the bond can be written O4–H…O$_{W539}$. Case 2: the H atom is from the water, W3, so that O4 is O2– and the bond can be written as O4…H–O$_{W539}$ (Supplementary Fig. 1). Using these two cases, we investigated whether formation of the short H-bond between O4 and W539 is energetically possible in the S$_1$ to S$_1$ states of the high oxidation state model.

In the low oxidation state model these Mn valence states correspond to the S$_1$–S$_1$ states$^{35}$ and this option was also investigated (Table 1), however in the present study, if not otherwise specified, the S-states given refer to the high oxidation state model. For discussions of the Mn oxidation state of the low oxidation state model$^{46}$, see Supplementary Discussion.

**Case 1.** O4 is an H-bond donor (O4–OH$^-$) to W539 ("pre-PT" H-bond pattern). In S$_0$ ((Mn1, Mn2, Mn3, Mn4) = (III, IV, III, III)), QM/MM calculations reproduced a short H-bond distance of ∼2.5 Å (2.55 Å, Table 1) for O4–O$_{W539}$ only when O4 was OH$^-$ and donated an H-bond to W539 ("pre-PT" pattern in Fig. 2, Table 1). In S$_1$, the energy profile of the O4–O$_{W539}$ bond (Fig. 3a) resembles an energetically unstable single-well H-bond (Supplementary Fig. 2). These results indicate that matching of the pK$_a$ values of the donor (that is, O4, Supplementary Fig. 1) and acceptor (that is, W539) moieties occurs in S$_1$. Due to the presence of the single-well H-bond (for example, ref. 47), the "pre-PT" H-bond pattern (Fig. 2) was energetically unstable in S$_1$ (discussed later). Note that a single-well H-bond has its energy minimum only at the O–O distance of ∼2.5 Å (for example, not 2.6 or 2.7 Å (ref. 43)).

**Case 2.** W539 is an H-bond donor to O4 (O4 = O2–) ("post-PT" H-bond pattern). In the over-reduced state, S$_{-1}$, QM/MM calculations also reproduced the short H-bond distance of ∼2.5 Å for O4–O$_{W539}$ (Table 1) but only when O4 was O2– and when it accepted an H-bond from W539 ("post-PT" pattern in Fig. 2).

**PT from OH$^-$ at O4 along the O4-water chain.** In the QM/MM calculations, OH$^-$ at O4 was stable as an H-bond donor to W539 in S$_0$ and lower S states (Fig. 3). However, in the S$_1$ state, OH$^-$ at O4 was energetically unstable due to formation of the single-well H-bond, leading to the release of a proton from OH$^-$ at O4 and the formation of O$_2^-$ at O4. Remarkably, the released proton was...
stabilized at W1047 in the form of H$_3$O$^+$, which is 13.5 Å away from O4 (Fig. 1). Overall, the pre-PT pattern (Fig. 2) in the initial state completely transformed into the post-PT pattern (Fig. 2) as a result of PT.

The short O4–O$_{W393}$ in the pre-PT conformation lengthened to $\sim$2.58 Å in the post-PT pattern in S$_0$ or S$_1$ (Table 1) from 2.50 Å in the 1.9-Å structure. This longer H-bond in S$_0$ or S$_1$ is consistent with O4–O$_{W393}$ distances of 2.59 Å in both PSI1 stabilized at W1047 in the form of H$_3$O$^+$, which is 13.5 Å away from O4 (Fig. 1). Overall, the pre-PT pattern (Fig. 2) in the initial state completely transformed into the post-PT pattern (Fig. 2) as a result of PT.

The short O4–O$_{W393}$ in the pre-PT conformation lengthened to $\sim$2.58 Å in the post-PT pattern in S$_0$ or S$_1$ (Table 1) from 2.50 Å in the 1.9-Å structure. This longer H-bond in S$_0$ or S$_1$ is consistent with O4–O$_{W393}$ distances of 2.59 Å in both PSI1 stabilized at W1047 in the form of H$_3$O$^+$, which is 13.5 Å away from O4 (Fig. 1). Overall, the pre-PT pattern (Fig. 2) in the initial state completely transformed into the post-PT pattern (Fig. 2) as a result of PT.

![Figure 1](image.jpg)
monomers in the recent radiation-damage-free PSII crystal structure obtained using the X-ray free electron laser (Supplementary Table 1)\textsuperscript{36}. These results seem to correspond to a situation in which the free electron laser structure\textsuperscript{36} has the S\textsubscript{0} or S\textsubscript{1} state in which the O\textsubscript{4} is deprotonated, while in the earlier conventional X-ray diffraction structure (1.9-Å structure)\textsuperscript{14} the Mn\textsubscript{4}CaO\textsubscript{4} is in a more reduced state and exhibiting a single-well H-bond between O\textsubscript{4} and OW\textsubscript{539}. In the 1.9-Å structure, W\textsubscript{1047} has unusually short H-bonds, OW\textsubscript{1047}–OW\textsubscript{545} (2.50 Å) and OW\textsubscript{1047}–OW\textsubscript{399} (2.52 Å)\textsuperscript{14}. Intriguingly, the short O–O distances were reproduced only when we assumed the post-PT H-bond pattern of the O4-water chain (2.46–2.54 Å, Table 1 and Supplementary Table 2). In general, typical H\textsubscript{2}O\textsuperscript{−}H\textsubscript{3}O\textsuperscript{+} (that is, the Zundel cation) has an unusually short O–O distance of \( \sim 2.4 \) Å (refs 39,40). Therefore, the short H-bond distances of W\textsubscript{1047} in the 1.9-Å structure imply that W\textsubscript{1047} is capable of forming H\textsubscript{3}O\textsuperscript{+}. Remarkably, the X-ray free electron laser structure\textsuperscript{36} also has unusually short H-bonds at the corresponding positions (Supplementary Table 1), a further indication that the X-ray free electron laser structure represents the post-PT state. The presence of these single-well H-bonds indicates that local movement of a proton within the O\textsubscript{4}–OW\textsubscript{539} bond of \( \sim 2.5 \) Å results in a sequential downhill PT from O\textsubscript{4} to W\textsubscript{1047} over a distance of 13.5 Å (Fig. 2 and Supplementary Fig. 3).

A working model. The results indicate that PT occurs through the O\textsubscript{4}-water chain and thence towards the luminal bulk surface via PsbU (Supplementary Discussion). This PT reaction is specifically associated with the S\textsubscript{0}–S\textsubscript{1} transition. This is supported by the following arguments.

The O\textsubscript{4}-water chain is the H-bond network in the 1.9-Å structure\textsuperscript{14} that is directly linked with the O atoms of the Mn\textsubscript{4}CaO\textsubscript{4} cluster (via the O\textsubscript{4}–OW\textsubscript{539} bond). The energy barrier for PT along the O\textsubscript{4}–OW\textsubscript{539} bond is clearly the lowest among all the possible Mn\textsubscript{4}CaO\textsubscript{4} deprotonation sites in the 1.9-Å structure\textsuperscript{14}. This should contribute to a smaller activation energy for deprotonation, a property consistent with the known characteristics of the S\textsubscript{0}–S\textsubscript{1} transition, which includes its rate...
Energetics of proton release from O5. In the present work we also analysed the energy profiles of the PT along the O5 path that proceeds from O5 to D1–Asp61 via the water molecules, W2, W446 and W442 (Fig. 5). W2 is the only possible proton acceptor from OH– at O5 when the original geometry of the W446 and W442 (Fig. 5). W2 is the only possible proton that proceeds from O5 to D1–Asp61 via the water molecules, W2, also analysed the energy profiles of the PT along the O5 path (Fig. 5), suggesting that a sequential downhill PT from this step occurs relatively slowly (tens of microseconds21). The remarkable linear, eight-water molecular chain acting as Grothuss-like proton conduit, which is reported here, might be expected to work with very fast kinetics. However, proton release on this step occurs relatively slowly (tens of microseconds21). This is easily understandable since the electron transfer step occurs before H+ release and is thus rate-limiting21.

Figure 3 | The energy profiles along the PT coordinate for the O4–OW539 bond. (a) pre-PT and (b) post-PT in S1 (blue solid curve), S0 (black dotted curve) and S−1 (blue thin solid curve). For comparison, the energy minimum in the O4 moiety was set to zero for all S states.

Alternative O5 deprotonation models. Although some studies have suggested or assumed that O5 (= OH−) deprotonation occurs on the S0–S1 transition33–35, it should also be noted that O5 has no direct-H-bond partner in the original geometry of the 1.9-Å structure14 (Fig. 7) and in the free electron laser structure36. Recent QM/MM studies by Pal et al.34 also demonstrated that OH− at O5 has no direct-H-bond partner in their S0 model; this implies that the ‘energy barrier’ for PT with O5 must be high. Indeed, Pal et al. demonstrated that O5 deprotonation
only occurred when using ’a small model of the oxidized S0’ state’ (that is, not ’the QM/MM S0 model’, see supplementary Fig. 7b in ref. 34), in line with our conclusions.

Siegbahn put forward a model in which O5 (= OH−) deprotonation occurred on the S0–S1 transition by assuming an additional water molecule near O5 in density functional theory (DFT) calculations35, a water molecule that is not visible not only in the original 1.9-Å structure14 but also in the recent radiation-damage-free PSII crystal structure36. This extra water molecule was positioned so that it could accept an H-bond from O5 for modelling the S0 and S1 transition. Furthermore, the O5 deprotonation model also included a significant structural modification in the vicinity of the H-bond partner of O4, that is, W539, and the entire O4-water chain were also absent in the earlier model35. These changes and omissions are expected to affect the resulting energy of the system in that model.

Overall then based on the current data, it seems clear that the deprotonation of a OH− at O5, as suggested earlier34,35, is less likely than deprotonation of OH− at O4, as suggested in the present work.

Deprotonation of the substrate oxygen and protonation of O4. It has been proposed that the exchangeable μ-oxo bridge is likely to be O5 (refs 24,29,33,52; see also relevant articles19,20 published before the detailed 1.9-Å structure14), and thus O5 is a plausible candidate for the slow exchanging substrate water molecule Ws.
(ref. 53). This is not incompatible with the presence of protonated O4 in S0: it is possible that the liberation of O2 on formation of S0 is associated with the release of a proton from the substrate ending up on μ-oxo bridge O4. A similar protonation of a μ-oxo bridge on liberation of O2 has been proposed in manganese catalases34.

The O4-water chain and the low S0/S1 redox potential. The conversion of the S0 state into the S1 state due to its oxidation by TyrD* showed that the S0/S1 redox potential is lower than that of TyrD redox potential56, the latter was estimated to be ~720–760 mV (refs 57,58). Given that all the S0 present is converted to S1 by TyrD*, the S0/S1 redox potential can be taken to be ≤700 mV assuming the estimates for TyrD redox couple are reasonable. In contrast, the S1/S2 and S2/S3 redox potentials are clearly higher since they oxidize TyrD* and they have been suggested to be ~900–950 mV (ref. 58). Thus, the barrier-less deprotonation and the subsequent downhill PT reaction occurring on the S0–S1 transition may contribute to the uniquely low redox potential of this redox couple, compared with those of the other S-state transitions. As demonstrated by Warshel and coworkers47,51, low-barrier H-bonds (including single-well H-bonds), where the pKₐ difference for the donor and acceptor moieties is nearly zero (that is, less polarized), are particularly unstable in polar protein environments. An O4–Oₜ₅₃₉ H-bond will become a very unstable low-barrier H-bond upon oxidation to S1, resulting in the release of the proton via the O4-water chain (Table 1). This is consistent with the observation that S1 does not spontaneously back-react to form S0 (ref. 59). PT may proceed through different pathways depending on the S-state transitions5–7. Intriguingly, it has been reported that the rate constant for the S0–S1 transition is essentially pH-independent, whereas for S2–S3 transition is pH-dependent1,48,49. Ionizable groups are more likely to be involved in a PT pathway for a pH-dependent process than a pH-independent process; this fits the suggestion that the unchanged O4-water chain may be active in the S0–S1 transition.

Changes in Mn–Mn distances by Mn oxidation/deprotonation. On proton release from O4, the Mn ion undergoing oxidation was Mn3 (Fig. 8). We note that the Mn undergoing oxidation is unlikely to be Mn4 because it appears to be the most reduced site, that is, it has the highest potential (for example, refs 29 and 60). Our results suggest that on formation of S1, oxidation of Mn3 from the III valence to IV favours the release of the proton from OH– at O4 (Fig. 8). From ENDOR, electron paramagnetic resonance (EPR) and simulation of the EXAFS, it has been proposed that a Mn–Mn distance of ~2.85 Å in the S0 state is decreased to ~2.7 Å in the S1 state (2.83–2.73 Å (ref. 16), 2.85–2.72 Å (ref. 19) or 2.85–2.75 Å (ref. 20)). In the present calculations, Mn3–Mn4 was the only distance that was decreased by ~0.1 Å from S0 to 2.70 Å in S1 (Table 2 and Supplementary Table 4). Note that the corresponding change was 2.90, a slightly longer distance, to 2.71 Å when O5 was assumed to be the deprotonation site34. These results suggest that the decreased Mn–Mn distance reported in EXAFS studies16–21 corresponds to the Mn3–Mn4 distance, which is decreased by deprotonation of O4 in the S0–S1 transition.

Mn3–Mn4 is a key distance that can allow us to evaluate the reduction state of PSII crystal structures with respect to geometries obtained from ENDOR, EPR or simulation of the EXAFS16–21 in both S0 and S1. The Mn3–Mn4 distance of 2.87 Å in the free electron laser structure36, which was reproduced (2.91 Å with OH– at O5) in S1) in QM/MM studies by Shoji et al.61 is close to the distance of S0 (~2.85 Å), not the distance of S1 (~2.73 Å), obtained from ENDOR, EPR and EXAFS. This is in line with the presence of significant amount of the reduced S0 state suggested from recent QM/MM studies by Asherka et al.38. It seems likely that the geometry of the free electron laser structure does not represent a pure S1 state detected ENDOR, EPR and EXAFS16–21 and is probably in a more reduced state.

An argument for the presence of a hydroxide ion at O5 for the free electron laser structure proposed by Suga et al.36 was the observation of significantly lengthened distances between O5 and the adjacent Mn ions. The presence of a hydroxide ion at O5 was considered likely because recent QM/MM studies by Shoji et al.61 have reproduced two of the three significantly lengthened distances between O5 and the adjacent Mn ions, Mn1–O5 (2.70 Å (ref. 36) and 2.73 Å (ref. 61)) and Mn4–O5 (2.33 Å (ref. 36) and 2.34 Å (ref. 61)), by assuming OH– at O5 in S1 (ref. 61). However, it should also be noted that one of the three significantly lengthened distances in the free electron laser structure, Mn3–O5, could not be reproduced in this study61; in fact Mn3–O5 was significantly shortened in the QM/MM geometry (2.20 Å (ref. 36) to 1.96 Å (ref. 61)). Indeed, the 1.9 Å structure has an even longer Mn3–O5 distance of 2.38 Å (ref. 14). Notably, a similar Mn3–O5 distance of 2.37 Å was obtained, assuming reduced Mn(II) for Mn4 in DFT studies by Petrie et al.62.

These features of the free electron laser structure, (i) the lengthened Mn3–Mn4 distance with respect to S0 of ENDOR, EPR and EXAFS16–21 and (ii) the lengthened Mn3–O5 distance with respect to the QM/MM geometry (S1 with OH– at O5 (ref. 61)), suggest that Mn₃CaO₃ of the free electron laser structure is probably reduced and cannot be simply explained as representing a single oxidation (and protonation) state.
Table 2 | Mn–Mn distances (Å).

| Mn1-Mn2 | 3.36 | 3.28 |
| Mn1-Mn3 | 2.86 | 2.70* |
| Mn1-Mn4 | 4.74 | 4.81 |

*Essentially consistent with the value of 2.71 Å in S1 (O4 and O5 – O6 – O7) in other studies (for example, ref. 34).

Discussion

Based on the findings reported here, we are able to propose a mechanism for PT along the O4-water chain. (Note that the role of the O4-water chain as a proton channel as proposed here does not exclude a role as a substrate channel on other steps of the cycle.) On the S0–S1 transition deprotonation of a μ-hydroxo group at the O4 position occurs due to oxidation of Mn3(III) to Mn3(IV) (Fig. 8). This deprotonation results in a decrease in the Mn3-Mn4 distance from 2.86 to 2.70 Å (Table 2 and Supplementary Table 4); a similar decrease in the Mn–Mn separation has been reported in ENDOR, EPR or EXAFS studies16,19,20. The nature of the O4 water chain, being composed exclusively of water molecules, is consistent with and may explain the pH-independence of PT in the S0–S1 transition21,48,49. At the start of the O4-water chain, the proton in the barrier-less O4–OW39 H-bond is likely to move away from the positively charged S1 when it forms. Thus, the O4–OW39 H-bond is energetically less stable in S1, resulting in the sequential (Fig. 2) and downhill (Fig. 4) PT reaction. The presence of the O4-water chain may explain why formation of the S1 state is less inhibited by Cl– depletion63. It may also explain the apparently irreversible S0–S1 step55 and the redox potential of S0/S1 being less inhibited by Cl– depletion63. It may also explain the apparently irreversible S0–S1 step55 and the redox potential of S0/S1 being less inhibited by Cl– depletion63.

Methods

Coordination and atomic partial charges. The atomic coordinates of PSII were taken from the X-ray structure of PSII monomer unit ‘A’ of the PSII complexes from Thermosynechococcus vulcanus at a resolution of 1.9 Å (Protein Data Bank (PDB) code, 3ARC)14. Hydrogen atoms were generated and energetically optimized with CHARMM65, whereas the positions of all non-hydrogen atoms were fixed, and all titratable groups were kept in their standard protonation states (that is, acidic groups titratable groups were kept in their standard protonation states (that is, acidic groups). We employed the electrostatic embedding QM/MM method to calculate the Pt potentials of water molecules (including the ligands), water molecules shown in Fig. 1, side-chains of D1-Asp61, D1-Asn338, D2-Asn350, and CP43-Thr335, and backbones of D1-Asp61, D1-Asn335, D1-Ala336, D2-Asn350 and CP43-Gly338, whereas other protein units and all cofactors were approximated by the MM force field. To analyse all of the H-bonds along the O4-water chain (Fig. 1) in the pre-PT conformation, a slightly smaller QM region was defined as the (Mn3CaO) cluster (including the ligands), water molecules shown in Fig. 1 and the side-chain of CP43-Thr335 for efficiency (small QM). The two QM regions did not essentially alter the results and conclusions. The resulting geometries were essentially identical regardless of the size of the QM region (Supplementary Tables 2 and 4). The results obtained on the basis of the large QM region were described in the main text. To analyse all of the H-bonds along the O5 path (Fig. 1) in the pre-PT–like conformation, the QM region was defined as the (Mn3CaO, cluster (including the ligands), water molecules of W1, W2, W3, W4, W539, W538, W446, and W442, side-chains of D1-Asp61, D1-Asn1, D1-Ala336, and D2-Lys317 and the chloride ion (Cl–1)). To analyze H-bond patterns of the O5 and W2 moiety (Fig. 7), we used the QM region same as that in ref. 68. The geometries were refined by constrained QM/MM optimization. Specifically, the coordinates of the heavy atoms in the surrounding MM region were fixed at their original X-ray coordinates, while those of the H atoms in the MM region were optimized using the OPLS2005 force field. All of the atomic coordinates in the QM region were fully relaxed (that is, not fixed) in the QM/MM calculation. Note that the resulting atomic coordinates of the QM region essentially did not alter upon full relaxation of the entire atoms (including heavy atoms) in the surrounding MM region68. The Mn3CaO cluster was considered to be functionally coupled; for example, the Eu spin S = 7 in S1 and the resulting Mn oxidation state (Mn1, Mn2, Mn3, Mn4) = (III, IV, IV, III) (see Supplementary Discussion for further details). The potential-energy profiles of H-bonds were obtained as follows: first, we prepared the QM/MM optimized geometry without constraints and used the resulting geometry as the initial geometry. The H atom under investigation was then moved from the H-bond donor atom (Odonor) towards the acceptor atom (Oacceptor) by 0.05 Å, after which the geometry was optimized by constraining the Odonor–H and H–Oacceptor distances. The energy of the resulting geometry was calculated. This procedure was repeated until the H atom reached the Oacceptor atom. See Supplementary Data 1 for the atomic coordinates of the QM region (that is, Mn3CaO). As discussed later, OH– at O5 has no direct H-bond in the protein environment of PSII. Thus, to analyse the PT from O5, we assumed W2 (O5…OW539 = 3.1 Å (ref. 14)) as the plausible proton acceptor, and analysed the energy profile by constraining the H–O5 distance. We also calculated the 1H NMR chemical shift for OH– at O5 (see Supplementary Methods and Supplementary Discussion).

References

1. Dau, H., Zaharieva, I. & Haumann, M. Recent developments in research on water oxidation by photosystem II. Curr. Opin. Chem. Biol. 16, 3–10 (2012).
2. Cox, N. & Messinger, J. Reflections on substrate water and dioxygen formation. Biochim. Biophys. Acta 1872, 1020–1030 (2013).
3. Schlodder, E. & Witt, H. T. Stoichiometry of proton release from the catalytic center in photosynthetic water oxidation. J. Biol. Chem. 274, 30387–30392 (1999).
4. Suzuki, H., Sugimura, M. & Noguchi, T. pH dependence of the flash-induced water oxidation by photosystem II. Biochim. Biophys. Acta 1411, 86–91 (1999).
5. Vrettos, J., Limburg, J. & Brudvig, G. W. Mechanism of photosynthetic water oxidation: combining biophysical studies of photosystem II with inorganic model chemistry. Biochem. Biophys. Acta 1503, 229–245 (2001).
6. Renger, G. Photosynthetic water oxidation to molecular oxygen: apparatus and mechanism. Biochim. Biophys. Acta 1503, 210–228 (2001).
7. Murray, J. W. & Barber, J. Structural characteristics of channels and pathways in photosystem II including the identification of an oxygen channel. J. Struct. Biol. 159, 228–237 (2007).
8. Mo, F. M. & Stryer, S. Access channels and methanol binding site to the CaMn4 cluster in photosystem II based on solvent accessibility simulations, with implications for substrate water access. Biochim. Biophys. Acta 1777, 140–153 (2008).
9. Vasiliev, S., Zaraiskaya, T. & Bruce, D. Exploring the energetics of water permeation in photosystem II by multiple stereored molecular dynamics simulations. Biochim. Biophys. Acta 1817, 1671–1678 (2012).
10. Ogata, K., Yuki, T., Hatakeyama, M., Uchida, W. & Nakamura, S. All-atom molecular dynamics simulation of photosystem II embedded in thylakoid membrane. J. Am. Chem. Soc. 135, 15670–15673 (2013).
11. Linke, K. & Ho, F. M. Water in photosystem II: structural, functional and mechanistic considerations. Biochim. Biophys. Acta 1837, 14–32 (2014).
12. Dinicke, A. & Ho, F. M. Perturbing the water cavity surrounding the manganese cluster by mutating the residue D1-valine 185 has a strong surrounding the oxygen water oxidation mechanism of photosystem II. Biochemistry 52, 6824–6833 (2013).
13. Umeno, Y., Kawakami, K., Shen, J.-R. & Kamiya, N. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature 473, 55–60 (2011).
14. Gatt, P., Petrie, S., Stranger, R. & Pace, R. J. Rationalizing the 1.9 Å crystal structure of photosystem II—a remarkable Jahn–Teller balancing act induced by its partial reactions. J. Am. Chem. Soc. 124, 14011–14018 (2002).
15. Gatt, P., Petrie, S., Stranger, R. & Pace, R. J. Rationalizing the 1.9 Å crystal structure of photosystem II—a remarkable Jahn–Teller balancing act induced by its partial reactions. J. Am. Chem. Soc. 124, 14011–14018 (2002).
16. Umena, Y., Kawakami, K., Shen, J.-R. & Kamiya, N. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature 473, 55–60 (2011).
16. Baldwin, M. J. et al. Structural and magnetic effects of successive protonations of oxo bridges in high-valent manganese dimers. J. Am. Chem. Soc. 116, 11349–11356 (1994).

17. Yano, J. et al. X-ray damage to the Mn₄Ca complex in single crystals of photosystem II: a case study for metalloprotein crystallography. Proc. Natl Acad. Sci. USA 102, 12047–12052 (2005).

18. Grabolle, M., Haumann, M., Müller, C., Liebisb, P. & Dau, H. Rapid loss of structural motifs in the manganese complex of oxygenic photosynthesis by X-ray irradiation at 10–300 K. J. Biol. Chem. 281, 4580–4588 (2006).

19. Robblee, J. H. et al. The Mn cluster in the S₀ state of the oxygen-evolving complex of photosystem II based on high resolution ENDOR and EPR spectroscopy. J. Am. Chem. Soc. 124, 7439–7471 (2002).

20. Kulik, L. V., Epel, B., Lubitz, W. & Messinger, J. Electronic structure of the Mn₄O₅Ca cluster in the S₀ and S₃ states of the oxygen-evolving complex of photosystem II based on high resolution ENDOR and EPR spectroscopy. J. Am. Chem. Soc. 129, 13421–13435 (2007).

21. Dau, H. & Haumann, M. The manganese complex of photosystem II in its reaction cycle? Basic framework and possible realization at the atomic level. Coord. Chem. Rev. 252, 273–295 (2008).

22. Krewald, V. et al. Metal oxidation states in biological water splitting. Chem. Sci. 6, 1676–1695 (2015).

23. Britt, R. D. et al. Recent pulsed EPR studies of the photosystem II oxygen-evolving complex: implications as to water oxidation mechanisms. Biochim. Biophys. Acta 1655, 158–171 (2004).

24. Rapatskyi, L. et al. Detection of the water-binding sites of the oxygen-evolving complex of photosystem II using W-band EPR electron double resonance-detected NMR spectroscopy. J. Am. Chem. Soc. 134, 16619–16634 (2012).

25. Messinger, J. Evaluation of different mechanistic proposals for water oxidation in photosynthesis on the basis of Mn₄O₅Ca structures for the catalytic site and spectroscopic data. Phys. Chem. Chem. Phys. 6, 4764–4771 (2004).

26. McConnell, I. L. et al. The affinities for the two substrate water binding sites in the O₂ evolving complex of photosystem II vary independently during S-state turnover. Biochemistry 39, 4399–4405 (2000).

27. Hillier, W., Messinger, J. & Wydrzynski, T. Kinetic determination of the fast exchanging substrate water molecule in the S₃ state of photosystem II. Biochemistry 37, 16908–16914 (1998).

28. Kusunoki, M. S₁-state Mn₄Ca complex of photosystem II exists in equilibrium between the two most-stable isomeric substates: XRD and EXAFS evidence. J. Photochem. Photobiol. B 104, 110–111 (2011).

29. Cox, N., Pantazis, D. A., Neese, F. & Lubitz, W. Biological water oxidation. Acc. Chem. Res. 46, 1588–1596 (2013).

30. Sprott, N. G. & Collinson, J. B. Identification and localization of the water molecules in the Mn cluster of photosystem II based on high-resolution ENDOR and EPR spectroscopy. J. Am. Chem. Soc. 118, 15651–15656 (1996).

31. McConville, L. et al. Investigation of the high-resolution crystal structure of photosystem II. J. Am. Chem. Soc. 134, 1504–1512 (2012).

32. Pal, R. et al. S-state model of the oxygen-evolving complex of photosystem II. Biochemistry 52, 7703–7706 (2013).

33. Sato, K. et al. Water oxidation mechanism in photosystem II, including oxidations, proton release pathways, O–O bond formation and O₂ release. Biochim. Biophys. Acta 1827, 1003–1019 (2013).

34. Suga, M. et al. Structure and magnetic effects of successive protonations of oxo bridges in high-valent manganese dimers. J. Am. Chem. Soc. 116, 11349–11356 (1994).

35. Saito, K., Ishikita, H. & Saito, K. Proton transfer reactions and hydrogen-bond networks in protein environments. J. R. Soc. Interface 11, 20130518 (2014).

36. Guévov, A. et al. Cyanobacterial photosystem II at 2.9 Å resolution and the role of the protein environment, lipids, channels and chlorophyll. Nat. Struct. Mol. Biol. 16, 334–342 (2009).

37. Warnesh, A. & Hansum, B. Studies on the reaction coordinates of the water oxidation in PS II membrane fractions from spinach. FEBS Lett. 299, 28–32 (1991).

38. Haumann, M., Bögershausen, O., Cherepanov, D., Ahlbrink, R. & Junge, W. Photosynthetic oxygen evolution: H/D isotope effects and the coupling between electron and proton transfer during the redox reactions at the oxidizing site of photosystem II. Photosynth. Res. 193, 193–208 (1997).

39. Stuebebrukov, A. A. Mechanisms of proton transfer in proteins: localized charge transfer versus delocalized soliton transfer. Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 79, 031927 (2009).

40. Schuster, C. N. & Warshel, A. The low barrier proton hydrogen bond (LBHB) proposal revisited: the case of the Asp...His pair in serine proteases. Proteins 55, 711–723 (2004).

41. Salston, A., Robertazzi, A. & Knapp, E. Oxygen-evolving Mn cluster in photosystem II: the protonation pattern and oxidation state in the high-resolution crystal structure. J. Am. Chem. Soc. 134, 7442–7449 (2012).

42. Hiller, W. & Wydrzynski, T. The affinities for the two substrate water binding sites in the O₂ evolving complex of photosystem II vary independently during S-state turnover. Biochemistry 39, 4399–4405 (2000).

43. Hillier, W., Messinger, J. & Wydrzynski, T. Kinetic determination of the fast exchanging substrate water molecule in the S₃ state of photosystem II. Biochemistry 37, 16908–16914 (1998).

44. McConnell, I. L. et al. The affinities for the two substrate water binding sites in the O₂ evolving complex of photosystem II vary independently during S-state turnover. Biochemistry 39, 4399–4405 (2000).

45. SCHUBERT, V. et al. & ETZNER, D. Theoretical studies of the damage-free S₁ structure of the O₂-evolving system in chloroplasts by thylakoid components. Biochim. Biophys. Acta 764, 194–202 (1984).

46. Styring, S. & Renger, A. W. In the oxygen-evolving complex of photosystem II the S₃ state is oxidized to the S₄ state by D⁺ (signal IIslow). Biochemistry 26, 2401–2405 (1987).

47. Boussac, A. & Etienne, A. L. Midpoint potential of signal II (slow) in Tris-washed photosystem-II particles. Biochim. Biophys. Acta 766, 576–581 (1984).

48. Vass, I. & Styring, S. pH-dependent charge equilibria between tyrosine-D and the S₁ states in photosystem II. Estimation of relative midpoint redox potentials. Biochemistry 30, 830–839 (1991).

49. Kok, B., Forbush, B. & McGloin, M. Cooperation of charges in photosynthetic O₂ evolution–I A linear four step mechanism. Photochem. Photobiol. 11, 457–475 (1970).

50. Kurasheg, Y., Chao, G. Z. & Yanai, T. Entangled quantum electronic wavefunctions of the Mn₄Ca₃O cluster in photosystem II. Nat. Chem. 5, 660–666 (2013).

51. Shoji, M. et al. Theoretical studies of the damage-free S₁ structure of the CaMn₄O₅ cluster in oxygen-evolving complex of photosystem II. Chem. Phys. Lett. 623, 1–7 (2015).

52. Petrie, S., Pace, R. J. & Stranger, R. Resolving the differences between the 1.9 Å and 1.95 Å crystal structures of photosystem II: a single proton relocation defines two tautomeric forms of the water-oxidizing complex. Angew. Chem. Int. Ed. 54, 7120–7124 (2015).

53. Whittaker, M. M. & Whittaker, J. W. The oxidized (3,3) state of manganese catalase. Comparison of enzymes from Thermus thermophilus and Lactobacillus plantarum. Biochemistry 38, 9126–9136 (1999).

54. Vermaas, W. F. J., Renger, G. & Doihant, G. The reduction of the oxygen-evolving system in chloroplasts by thylakoid components. Biochim. Biophys. Acta 764, 194–202 (1984).

55. Brooks, B. R. CHARMM: a program for macromolecular energy minimization and dynamics calculations. J. Comput. Chem. 4, 187–217 (1983).

56. MacKerrill, I. A. et al. All-atom empirical potential for molecular modeling and dynamics studies of proteins. J. Phys. Chem. B 102, 3586–3616 (1998).

57. QSite, version 5 & Schrodinger, LLC. New York, NY, 2012.

58. Saito, K. & Ishikita, H. Influence of the Ca²⁺ ion on the Mn₄Ca₃O cluster and the H-bond network arrangement in photosystem II. Biochim. Biophys. Acta 1837, 159–166 (2014).
Acknowledgements

This research was supported by the JST PRESTO programme (K.S.), JSPS KAKENHI (22740276 and 26800224 to K.S. and 15H00864, 25107517, 26105012 and 26711008 to H.I.), Tokyo Ohka Foundation for The Promotion of Science and Technology (H.I.), and Biotechnological and Biological Sciences Research Council Grant (BB/K002627/1 to A.W.R.). A.W.R. is the recipient of a Wolfeon Merit Award of the Royal Society.

Author contributions

H.I. designed research; K.S. and H.I. performed research; K.S., A.W.R. and H.I. analysed data; and A.W.R. and H.I. wrote the paper.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

How to cite this article: Saito, K. et al., Energetics of proton release on the first oxidation step in the water-oxidizing enzyme. Nat. Commun. 6:8488 doi: 10.1038/ncomms9488 (2015).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/