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Mechanism of proton-coupled quinone reduction in Photosystem II

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Photosystem II uses light to drive water oxidation and plastoquinone (PQ) reduction. PQ reduction involves two PQ cofactors, QA and QB, working in series. QA is a one-electron carrier, whereas QB undergoes sequential reduction and protonation to form QBH, QBH, exchanges with PQ from the pool in the membrane. Based on the atomic coordinates of the Photosystem II crystal structure, we analyzed the proton transfer (PT) energetics adopting a quantum mechanical/molecular mechanical approach. The potential-energy profile suggests that the initial PT to QA+ occurs from the protonated, D1-His252 to QA+ via D1-Ser264. The second PT is likely to occur from D1-His215 to QBH via an H-bond with an energy profile with a single well, resulting in the formation of QAH, D1-His215 anion. The pathway for reproto

The core of the Photosystem II (PSII) reaction center is composed of D1/D2, a heterodimer of protein subunits containing the cofactors involved in photochemical charge separation, quinone reduction, and water oxidation. These reactions are driven by light absorption by pigments absorbing around 680 nm (P680). P680 is composed of four chlorophyll a (Chl) molecules, P668, Chl2, Chl2, and two phytochrome α molecules (PhoD1/PhoD2). Excitation of P680 initially leads to the formation of a charge separated states, with the Chl2, Pheo1, and Pheo1 – state dominating. After a short time the secondary radical pair, [P668/PhoD2]+, is formed in nearly all centers. This state is stabilized by electron transfer to the first quinone, QA, and by electron donation from a tyrosine residue, D1-Tyr160 (TyrZ), to P668. Qa then oxidizes the Mn4CaO5 cluster, which catalyzes the subsequent water splitting reaction. QA2+ acts as a one-electron redox couple, accepting electrons from PhoD2+ and donating to the second quinone, QB, without undergoing protonation itself. In contrast, QA reduction involves two consecutive one-electron reduction reactions with a series of associated proton uptake reactions (reviewed in 1–6).

QB is located near the nonheme Fe2+ center and the ligand to the Fe2+, D1-His215, donates an H-bond to the Qb carbonyl O atom that is nearer to the Fe complex (O(Fe-O(QB))). The Qb carbonyl O atom distal to the Fe complex (O(Fe-O(QB))) accepts an H-bond from D1-Ser264, which itself accepts an H-bond from D1-His252 (Fig. 1), which is located on the protein surface in contact with the aqueous medium (5–9). It is known that QA+ formation is linked to proton uptake (10, 11) and comparisons with the structure of the bacterial reaction center led to the first suggestion that the D1-His252 was the group undergoing protonation in response to QA+ formation (12). In theoretical studies, it has been proposed that proton uptake by D1-His252 causes reorientation of the hydroxyl group of D1-Ser264 toward the distal Qb carbonyl group and stabilizes QA+, facilitating the initial electron transfer (ET) from QA to QB (8).

Similar quinone reduction reactions occur in photosynthetic reaction centers from purple bacteria, which are thought to share a common ancestor with PSII (4, 13, 14). Notably, D1-Ser264 and D1-His252 in PSII are equivalent to Ser-L223 and Asp-L213, respectively, in the reaction center from Rhodobacter sphaeroides. Purple bacterial reaction centers contain an additional globular subunit, the H-subunit, that covers the quinone/Fe surface. Consequently, longer proton pathways exist to allow protons from the medium to reach the Qb binding site. The residues involved in this proton transfer (PT) pathway include Glu-L212, Asp-L213, Ser-L223, Asp-M17, and several residues of the H-subunit (15, 16). Glu-L212 is protonated in response to the formation of QA+ on the first turnover. When Asp-L213 is protonated (via the same proton pathway), reorientation of the Ser-L223 hydroxyl group occurs and an H-bond forms between Ser-L223 and O(QB(distal)) (17–20). Protonation of QA+ to form QA+ happens at O(QB(distal)) via Ser-L223 and Asp-L213 upon formation of the QA+ state. Electron transfer then takes place leading to formation of QA+, which then undergoes protonation at O(QB(proximal)) forming QBH2, with the proton from Glu-L212 (15). For PSII the current state of knowledge lacks these details, but several of the reactions are considered to be similar (reviewed in 4–6, 21). There are, however, several obvious structural differences between the two systems that must result in mechanistic differences. Glu-L212, for example, which appears to be a prerequisite for the second protonation step in purple bacterial reaction centers (RCs) (15, 16), is replaced with Ala in PSII. Another potentially important difference is the presence of bicarbonate in PSII instead of the Glu-M234 in purple bacterial RCs. This exchangeable carboxylic acid has been linked to several phenomena specific to PSII, notably (i) slowed electron transfer when bicarbonate is replaced with other carboxylic acids, (ii) a specific EPR signal from the semiquinone–iron complex, and (iii) redox activity of the nonheme iron (4–6, 21).

Here, we investigated formation of QA+ and QAH2 in the PSII protein environment, by adopting a large-scale quantum mechanical/molecular mechanical (QM/MM) approach based on the crystal structure with resolution at 1.9 Å (9).

Results and Discussion

First Protonation Step: Conversion of QA+ to QA+ via D1-His215 and D1-Ser264. To elucidate how the conversion of QA+ to QA+ occurs, we analyzed the potential-energy profiles of the two

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H-bonds of QB

Because in previous electrostatic calculations, D1-His252 became protonated upon formation of the QB* state, we also assumed the presence of protonated D1-His252 and the QB* state in the present QM/MM calculation. In general, serine is unlikely to deprotonate. However, in the QB* state, the potential-energy profile indicates that a PT from D1-Ser264 to OQB(distal) occurred very easily in an energetically downhill process (Fig. 2). This reaction was accompanied by a concerted PT from protonated D1-His252 to D1-Ser264, resulting in the formation of QB(distal)H+, deprotonated (neutral) D1-His252, and reoriented D1-Ser264 (Fig. 1, first and second panels). The QM/MM-optimized geometry indicates that the two H-bonds of D1-Ser264, OQB(distal)...H–OD1-Ser264 (2.48 Å) and OD1-Ser264...H–NδD1-His252 (2.51 Å), are unusually short, especially in the QB* state (Table 1). The two short H-bonds were only present before the initial PT occurred, but they lengthened (to 2.73 and 2.67 Å, respectively) immediately after PT had occurred. Therefore, the presence of an unusually short H-bond indicates that PT between the donor and acceptor moieties is about to occur.

In contrast, the potential-energy profile of the OQB(proximal)...H–NδD1-His215 (Fig. 2) resembles that of a standard asymmetric double-well H-bond (22) (Fig. S1), suggesting that the first PT from D1-His215 to OQB(proximal) is an energetically uphill process. This is primarily because proton release from the singly protonated (neutral) His (pKₐ ~14 for imidazole) (23) is unfavorable, unlike the doubly protonated (positively charged) His, for which the pKₐ ~7. Although the pKₐ for neutral His is expected to be lowered to some extent by the positive charge and environment around the iron (see below), it is still likely to be relatively high and thus unfavorable on this step.

The potential-energy profiles in Fig. 2 indicate that the initial PT occurs more favorably when Qₐ* is present compared with the neutral Qₐ state. This is similar to the situation occurring in the purple bacterial RC, where Qₐ* is unprotonated on the first flash and the Qₐ+ to QₐH⁺ step only occurs when the [Qₐ–Qₐ+] state is formed, before the second electron transfer step (15, 16).

Overall, the results suggest the following model for the first protonation: Protonation of QB* to QBH⁺ primarily occurs at OQB(distal), this occurs as a result of concerted PT from protonated D1-His252 to D1-Ser264, and reoriented D1-Ser264 (Fig. 1, first and second panels). The QM/MM-optimized geometry indicates that the two H-bonds of D1-Ser264, OQB(distal)...H–OD1-Ser264 (2.48 Å) and OD1-Ser264...H–NδD1-His252 (2.51 Å), are unusually short, especially in the QB* state (Table 1). The two short H-bonds were only present before the initial PT occurred, but they lengthened (to 2.73 and 2.67 Å, respectively) immediately after PT had occurred. Therefore, the presence of an unusually short H-bond indicates that PT between the donor and acceptor moieties is about to occur.

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Fig. 2. Potential-energy profiles of the H-bond donor–acceptor pairs: (Right) H-bond between D1-Ser264 and the distal QB carbonyl (purple, in the neutral QB state, and blue, in the reduced QB state, curves); (Left) H-bond between D1-His215 and the proximal QB carbonyl (red curves). At each point, all of the atomic coordinates in the QM region were fully relaxed (i.e., not fixed). Arrows indicate the directions of PT.
The signif

Table 1. H-bond distances in optimized geometries in the PSII protein environment near Q₈ systems (in Å)

<table>
<thead>
<tr>
<th>Q₈ state</th>
<th>Crystal</th>
<th>Q₈</th>
<th>Q₈⁺</th>
<th>Q₈H⁺</th>
<th>Q₈H²⁺</th>
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<td>neut.</td>
<td>neut.</td>
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<td>anion</td>
<td>neut.</td>
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<td>2.77</td>
<td>2.68</td>
<td>2.67</td>
<td>2.47⁺</td>
<td>2.55</td>
<td>2.77</td>
<td>2.62</td>
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<tr>
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<td>1.65</td>
<td>1.65</td>
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<td>1.77</td>
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<td>1.04</td>
<td>1.03</td>
<td>1.15</td>
<td>1.53</td>
<td>1.02</td>
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<tr>
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<td>0.21</td>
<td>0.22</td>
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</table>

H-bond donor-acceptor distances of <∼2.5 Å are indicated in bold. H-bond donor-acceptor distances are shaded in gray. Crystal = 1.9-Å structure (9) (PDB entry 3ARC), anion, doubly deprotonated (negatively charged) His; prot., doubly protonated (positively charged) His; neut., singly protonated (either at Nα or Nε) His.

*2.54 Å when the bicarbonate (HCO₃⁻) ligand was replaced with carbonate (CO₃²⁻).

was found to be unusually short (2.47 Å) in the Q₈H⁻ state (Table 1).

Intriguingly, this distance is identical to that in the 1.9 Å structural model, PSII monomer unit (“A”) of the PSII complexes (9). The corresponding O₂B(prox.)...H-N₈D₁-H₂15 distances were found to be 2.77 Å in the Q₈ state and 2.68 Å in the Q₈⁺ state (Table 1), both being significantly longer compared with that in the Q₈H⁻ state. In addition, the corresponding O₆A-chain distance (O₂B(prox.)...N₅P₂O₂-H₂14) was 2.78 Å in the 1.9 Å structure (9) and ∼2.8-2.9 Å in the purple bacterial RC (16). Given that the O₆A geometry was less well defined than O₆A geometry in the 1.9 Å structure (9), the significance of the short O₂B(prox.)...N₅P₂O₂-H₂15 distance (2.47 Å) distance should be treated with caution. Furthermore, the Q₈H⁻ state is expected to be a short-lived intermediate and not a state that would be present and in PSII under normal circumstances. Nevertheless, more discussion of the possible redox states of Q₈ in the crystal structure is given in the SI Text.

In a typical H-bond with an O-O distance longer than ∼2.6 Å, an H-atom is located near the donor moiety owing to the larger pKw value of the donor moiety relative to the acceptor moiety (having an asymmetric double-well potential H-bond) (22) (Fig. S1). On the other hand, according to the classification of H-bonds by Jeffrey (25) or Frey (26), short H-bonds with O-O distances of 2.4-2.5 Å can be classified as single-well (ionic) H-bonds (22) (Fig. S1). Because O-N distances are generally greater than O-O distances, the O₂B(prox.)...N₅P₂O₂-H₂15 of 2.47 Å is an unusually short H-bond and may possess a single-well potential. Remarkably, the calculated potential-energy profile for the O₂B(prox.)...H-N₅P₂O₂-H₂15 in the Q₈H⁻ state resembled that of a barrierless single-well (ionic) H-bond, suggesting that the second PT can occur isomerically at O₂B(prox.) (Fig. 2). The significantly elongated H-N bond of D1-His215 (1.15 Å) in the Q₈H⁻ state implies that further migration of an H atom toward the acceptor O₂B(prox.) moiety (i.e., PT) can occur easily. Indeed, the single-well potential obtained for O₂B(prox.)...N₅P₂O₂-H₂15 is symmetric (Fig. 2), implying that the pKw difference (27) between D1-His215 deprotonation and Q₈H⁻ protonation is close to zero.

The pKw for the QH⁻ to QH²⁺ protonation for plastoquinone (PQ) is expected to be similar to that measured for ubiquinone in aqueous solution—i.e., 10.7, significantly higher than the pKw of 4.9 for the protonation of the semiquinone, Q⁺ to QH⁺ (18, 28). The pKw for deprotonation of a neutral His is expected to be similar to that for imidazole—i.e., ∼14 (23). In PSII, however, the ligation of D1-His215 to the positively charged Fe²⁺ should lower the pKw of neutral D1-His215. The pKw of the neutral His ligand to Fe²⁺ in the Rieske (2Fe-2S) cluster has been measured to be ∼12.5 rather than ∼14 (29, 30). The ligand environment of the Fe²⁺ in PSII is more positively charged than that in the Rieske cluster; thus, the pKw of neutral D1-His215 deprotonation is expected to be lower than ∼12.5. In agreement with this, FTIR studies have indicated that deprotonation of D1-His215 occurs in response to pH changes (31). Overall then the literature suggests that the pKw value of D1-His215 is likely to be close to that for the QH⁻ to QH²⁺ protonation, in accordance with the single-well potential obtained here (Fig. 2). For further details, see Table S1.

It has been proposed that Glu-L212 in purple bacterial RC from R. sphaeroides provides a proton to QH²⁺ (15, 32, 33). This residue is 5.7 Å away from O₂B(prox.) (34), and it is not clear how this last protonation reaction occurs. One possibility is that the QH⁻ state is also protonated by the Fe-ligated imidazole (His-L190) and that Glu-L212 provides a proton to the deprotonated His residue (His-L190) facilitating the release of Q₈H² from the site. Such a scenario has been discussed (and disfavored) previously (15). Ionizable residues corresponding to Glu-L212 are absent near Q₈ in PSII. PSII may not require the corresponding residue, as the quinones are more exposed to the aqueous phase. Doubly deprotonated D1-His215 may be reprotonated via the bicarbonate ligand; indeed, a role for the bicarbonate in protonation of Q₈ has been considered for many years (21). The distance between O₂B(prox.) and Obicarbonate is 4.8 Å (9). Despite this long distance, rapid PT may be possible if water intermediate(s) were involved. In purple bacteria, water intermediates may also mediate PT from Glu-L212 to QH⁻, perhaps via the His-L190 anion.

H-Bond Pattern of the Tyrosine Residue Pair near the Bicarbonate Ligand. FTIR studies by Takahashi et al. have suggested that only one of the two tyrosine residues, either D1-Tyr246 or D2-Tyr244, provides an H-bond to bicarbonate (35). We investigated the influence of the H-bond pattern of these tyrosine residues on the stability of Q₈⁺ and Q₈++. To do this, we redefined the QM region such that it included Q₈, the nonheme Fe and its ligands, Q₈, D1-Tyr246 and D2-Tyr244. Note that in the calculations, in the absence of the PSII protein environment (i.e., in vacuum),
electrons were almost evenly distributed over QA and QB owing to the structural symmetry (Table S2).

When the tyrosine hydroxyl groups were oriented toward QA in the presence of deprotonated (neutral) D1-His252, QA* favored orientation (QA* favored orientation) was more pronounced and (QA*0.95 [Fe(His)](HCO3-) 0.67 [Qb*0.7]) with values of QA/ QB = 50/22 for electron distribution and 69/33 for spin distribution (Table S2; Fig. 3). On the other hand, when the tyrosine hydroxyl groups were oriented toward QB in the presence of protonated D1-His252 (QB* favored orientation), QB* formation was high ([QA*0.05 [Fe(His)](HCO3-) 0.30 [QB*0.64; QA/ QB = 84/84 for electron distribution and 8/98 for spin distribution]) (Table S2; Fig. 3). QA* formation was predominantly influenced by the protonation state of D1-His252 and orientation of D1-Ser264, as suggested in previous theoretical studies (8). On the other hand, donation of an H-bond from D1-Tyr246 to bicarbonate (i.e., QA* favored orientation) appears to play an important role particularly in the stability of QA*, because D1-His252/D1-Ser264-like residues are absent near QA. The orientation of the tyrosines may well be related to communication between the quinone sites via the H-bonding network of bicarbonate, gating, and/or protonation reactions during electron transfer (Fig. 3).

The two calculated conformations shown in Fig. 3 differ not only in terms of the orientations of D1-Tyr246 and D2-Tyr244 but also in terms of the orientation of bicarbonate, implying a link between the bicarbonate and the quinone redox states, as suggested by FTIR studies (31, 35). The two tyrosine residues are located in the n-de loop region (D1-225-250 and D2-224-248), which is believed to be crucial to the stability of the QA* state (36). The corresponding loop region is absent in the purple bacterial RC. Thus, the semiquinone stabilization mechanism, involving D1-Tyr246, D2-Tyr244, bicarbonate, and the rearrangement of the H-bond network, as suggested here, is only relevant to PSI.

Recently it was suggested that the ligation of the Fe2+ by bicarbonate changes during electron transfer, going from bidentate to monodentate upon formation of QA* and returning to the bidentate form upon electron transfer to QA (37). In ref. 37, a monodentate ligand yielded two significantly different Fe–O bicarbonate distances of 2.3 and 3.2 Å for QA* formation. Because the atomic coordinates were unavailable, we could not evaluate the specific models discussed in ref. 37, however we addressed the same question in our calculations using the most recent structure (9). We could not observe such a dramatic (~1 Å) change of the Fe–O bicarbonate bond. Our calculations showed that bicarbonate was clearly a bidentate ligand in both conformers irrespective of the reduction state of the two quinones: the two Fe–O bicarbonate distances were 2.21 and 2.29 Å in the presence of QA* and 2.27 and 2.32 Å in the presence of QB* (Fig. 3). We conclude that the formation of stable QA* or QB* states do not result in dramatic differences in the bicarbonate ligation such as those proposed by Chernev et al. (37). It seems possible that the changes in the environment of the nonheme Fe2+ reported by Chernev et al. using X-ray absorption spectroscopy could have resulted from the changes in the H-bond network associated with D1-Tyr246, D2-Tyr244, and the bicarbonate (Fig. 3). These results appear to fit better with FTIR studies by Takahashi et al. (35). A further argument against electron transfer-induced changes in the ligation of the nonheme Fe2+ comes from EPR studies. The EPR spectra from QA*–Fe2+ formed at room and cryogenic temperature and QA*–Fe2+ are all essentially the same (38, 39). A difference in the number of ligands to the Fe would be expected to result in more marked differences in these spectra.

Replacement of the Bicarbonate Ligand. Formate. The depletion of (b)carbonate or its substitution by formate results in a slowing of quinone reduction (6, 21). Recent EPR studies have suggested that formate inhibits release of QA2H2 (39). The PT mechanisms reported here suggest that the efficient release of QA2H2 requires the reprotonation of the anionic D1-His215. This process may involve bicarbonate, forming carbonate and releasing a proton to anionic D1-His215, as suggested here and earlier (21, 31). When bicarbonate is replaced with formate, the potential-energy profile indicates that the second protonation state is essentially unchanged and can occur isoenergetically at QA* (proximal) (Fig. S2). Note that the calculated QA*–Fe2+ distance was 2.49 Å in the QA* state with the formate ligand, which is essentially the same as that with bicarbonate ligand (2.47 Å, Table 1). In the EPR work, a new EPR signal was reported from the formate-inhibited enzyme when reduced by three electrons, attributed to QA*–Fe2+ in the presence of a two-electron reduced form of QB (39). In light of the present work, we suggest that this could correspond to QA*–Fe2+ QB2H with the anionic D1-His215.

Carbonate. Recently, the characteristic g-value of ~1.9 for the semiquinone and nonheme Fe complex in EPR spectroscopy (40) was re-investigated in theoretical simulations. On the basis of the simulations, it was proposed that the native ligand to the nonheme Fe was carbonate (CO32−) rather than bicarbonate (HCO3−) (41). In contrast, FTIR studies suggested that the bicarbonate ligand does not deprotonate even upon oxidation of the nonheme Fe (42). When the bicarbonate was substituted with fully ionized carbonate in our calculations, the QA H+ to QA2H2 protonation process became slightly energetically uphill and the QA*–Fe2+ state distance was 2.27 Å from the QA*–Fe2+ state (Table 1 and Fig. S2). These results suggest that the pKa of D1-His215 deprotonation was upshifted upon replacement of bicarbonate with carbonate and that the ligand plays a role in affecting the pKa of D1-His215, as proposed previously (31, 41).

We also calculated the charge distribution in the PSI protein environment with carbonate (QM region: QA, nonheme Fe, His and carbonate ligands, QB, D1-Tyr246, and D2-Tyr244). Starting with carbonate-ligated Fe2+ and two neutral quinones, the QM/MM calculations resulted in the oxidized Fe2+ and a neutral quinone (Table S1) QM/MM calculations for the carbonate-ligated Fe2+ and two neutral quinones resulted in oxidized Fe2+ and two reduced quinones. These results suggest that the fully ionized carbonate ligand forces Fe2+ to release an electron to one of the quinones. This does not reflect experimental findings, so these results argue against carbonate being a stable ligand to the iron and indicate another explanation must be found for the characteristic EPR spectra of the semiquinone iron signals seen in PSI. More discussion of the possible carbonate ligand is given in the SI Text.

Tyrosine Peroxide at D1-Tyr246. Possible Link with Photoinhibition, and a Water-Mediated H-Bond Network. In both of the PSI monomer units of the 1.9-Å structure, an elongation of density is clearly seen near the hydroxyl O atom of D1-Tyr246. In QM/MM calculations, atomic coordinates of a tyrosine peroxide (Tyr-OOH)
form (rather than a tyrosine with a water molecule) fitted to the density (Fig. 4). Root-mean-square deviation of the optimized coordinates for Tyr-OOH with respect to those in the 1.9-A structure is small (0.123 Å), even slightly smaller than that for Tyr-OOH (0.149 Å). A Tyr-OOH at this position may be relevant to the function of PSIII and is particularly intriguing in light of the literature: (i) Under strong light, the nonheme Fe$^{2+}$ is thought to be involved in the generation of hydroxyl radicals (OH*) via iron-peroxo intermediates (43, 44) and Tyr-OOH can be generated in the presence of OH* (45). (ii) The δ-de loop (D1-238–249 including D1-Tyr246) was proposed to be the first target for cleavage during photodegradation of D1 protein (46) and OH* may trigger this process (44). (iii) Spectroscopic studies suggest that the nonheme Fe undergoes an increase in its redox potential and a minor modification of the bicarbonate binding site during photodamage (47). These and other observations in the literature could be linked to Tyr-OOH formation. Although it is possible that D1-Tyr246 may become a peroxide under photoinhibitory conditions, its presence in the crystal structure could represent OH* generated by the X-ray beam (48). The absence of the peroxide-like density for its counterpart D2-Tyr244 and the presence in both monomers in the crystal structure suggests that peroxide generation specifically occurs at D1-Tyr246. The long distances (4.8/6.5 Å) between bicarbonate and D1-His215/QBH$^+$ imply that a direct PT would be kinetically unfavorable. QM/MM optimized geometries indicate that inclusion of water completes an H-bond network from bicarbonate to D1-Tyr246 (Fig. 4). There is, however, no resolved water molecule in this region in the 1.9 Å structure (9). We speculate that the proposed Tyr-OOH at D1-Tyr246 could have arisen from Tyr-OOH and a bridging water molecule. Photolytic-generation of Tyr-OOH at D1-Tyr246 is expected to remove the water molecule and disconnect the PT path between bicarbonate and D1-His215. The presence of Tyr-OOH specifically at D1-Tyr246 but its absence at its counterpart D2-Tyr244 on the QA side fits with the expectation that protons are excluded from the QA side, as it functions as a one-electron couple that does not show pH dependence (49).

**Working Model.** Based on the findings reported here and the literature discussed above, we are able to propose a mechanism for quinone reduction in PSII: (i) Protonation of D1-His252 occurs upon electron transfer from QA$^{•−}$ to QB$^+$ forming QBH$^+$. (ii) The presence of QA$^{•−}$ formed on the second turnover, triggers protonation of the QA$^{•−}$ from D1-Ser264 with the concerted arrival on the serine of a proton from the protonated D1-His252, leading to neutral histidine formation. (iii) As in the purple bacterial reaction center, QBH$^+$ may be transiently formed before the second electron arrives from QA$^{•−}$, forming QA$^{•}$H$^+$. (iv) QA$^{•}$H$^+$ has a single-well H-bond to the D1-His215 (ligand to the nonheme iron) and this favors the PT forming QAH$_2$, which remains H-bonded to the D1-His215 anion. (v) The release of QAH$_2$ is facilitated by reprotonation of D1-His215 anion and this may occur through water in the QA$_2$ site, which is part of an H-bonded network with the bicarbonate (a ligand to the Fe$^{2+}$) and groups exposed to the aqueous medium. (vi) The orientation of the tyrosine hydroxyl groups (e.g., D1-Tyr246) contributes to stabilizing reduced states of quinones. D1-Tyr246 may be involved in the H-bond network involving bicarbonate, water, D1-His215, and QAH$_2$. This working model should allow specific features to be tested by future experimentation.

**Computational Procedures**

**Coordinates 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 1.9-Å resolution (PDB code, 3ARC) (9). Hydrogen atoms were generated and energetically optimized with CHARMM (50), whereas the positions of all nonhydrogen atoms were fixed, and all titratable groups were kept in their standard protonation states (i.e., acidic groups were ionized and basic groups were protonated). For the QM/MM calculations, we added additional counter-ions to neutralize the entire system. Atomic partial charges of the amino acids were adopted from the all-atom CHARMM22 (51) parameter set. The atomic charges of Chl, Pheo, and quinones were taken from our previous studies on PSII (52). We considered the MnxCaO$_5$ cluster as the (O$_4$)$^{4−}$-(O$_5$)$^{5−}$ model (53) in the S1 state (see ref. 53 for the atomic coordinates and charges).

**QM/MM Calculations.** We used the electrostatic embedding QM/MM scheme, in which electrostatic and steric effects created by a protein environment were explicitly considered, and we used the Qsite (54) program code as used in previous studies (52). Owing to the large system size of PSII, we considered residues and cofactors in only the D1 and D2 subunits as the protein environment. We used the unrestricted density functional theory method with the B3LYP functional and LACVP** basis sets. To analyze the effects of the H-bond pattern near the bicarbonate-tyrosine moiety, the QM region was defined as (QA, QB, bicarbonate, Fe, D1-His215, D1-His272, D2-His215, D2-His268, D1-Tyr246, and D2-Tyr244), whereas other protein units and all cofactors were approximated by the MM force field. To analyze the H-bond potential energy profiles, the QM region was redefined as (QA, D1-His252, D1-Ser264, bicarbonate, Fe, D1-His215, D1-His272, D2-His215, and D2-His268). The tail of PQ was replaced with a methyl group at C11. As in a previous study (37), we assumed a high-spin state (S = 2) of Fe$^{2+}$ and set the spin multiplicity of the system to S = 2 in calculations for QA, QAH$_2$, and QAH$_3$, and S = 0/2 for QA$^{•−}$, QA$^{•}$H$^+$, and QA$^{•}$H$_2$.$^{•−}$. The geometries were re-optimized using QM/MM optimization. Specifically, the coordinates of the heavy atoms in the surrounding MM region were fixed to the original X-ray coordinates, whereas those of the H atoms in the MM region were optimized using the OPLS2005 force field. All of the atomic coordinates...


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**Supporting Information**

**Saito et al. 10.1073/pnas.1212957110**

**SI Text**

**Possible Redox States of QB in the Crystal Structure.** Before we dismiss the similarity between the calculated and measured distances of the QBH–His215 H-bond as coincidental, it is worth considering the possibility that the crystal structure does represent the QBH state. It is predicted that ~50% of the centers contain QBH in this kind of material when dark-adapted (1). X-ray–induced electrons are expected to reduce cofactors more efficiently than protein side-chains and significant structural changes can take place at 100 K (2). Thus, if QBH underwent a second reduction while in the beam, it would likely undergo protonation from the distal side H-bond from the protonated Ser/His pair (Fig. 2) and then approach the D1-His215, resulting in the shortened proximal H-bond, as predicted in the QM/MM model. The poorer resolution of QB compared with QA presumably reflects at least in part the distribution of redox states and structures expected for QB. It should also be noted that the other PSII monomer unit of the dimeric 1.9 Å structure (3) has an O_{QB(proximal)}–N\_D1-His215 distance of 2.62 Å, a distance identical to that calculated for the reduced QB state (Table 1). Here again the resolution is less than for QA, again presumably reflecting a mixture of conformations and redox states; in this case, however, the X-ray beam would have induced the one-electron reduction of the QB, forming QB\(_{\text{2+}}\). Further reduction of QB\(_{\text{2+}}\) in the beam is presumably inhibited because the lack of protonation linked changes at 100 K. This line of thought then allows for the possibility that the short distances associated with the QBH state could be formed in a significant fraction of the PSII in the crystal structure. Specific experiments need to be done controlling the redox state of QB in the crystals to test this.

**Residues That May Stabilize the Carbonate Ligand.** In ref. 4, the presence of the carbonate ligand was rationalized by its proximity with D2-Lys264 (4.4 Å) on the basis of the previous PSII crystal structure (5). However, the presence of the D1-Glu244, which is only 3.5 Å away from the (bi)carbonate ligand, does not seem to have been taken into account (figure 5 in ref. 4). The new structure (3) confirms that D1-Glu244, which is 3.3 Å away from the (bi)carbonate ligand, is ionized due to the salt-bridge formation with D2-Lys264 (3.3 Å from D1-Glu244), and that D2-Lys264 is slightly more distant from the (bi)carbonate ligand (5.0 Å). Hence, D2-Lys262 plays a role in stabilizing anionic D1-Glu244, which energetically favors bicarbonate over carbonate in the original geometry of the 1.9-Å structure (3). Nevertheless, if D2-Lys262 could approach the (bi)carbonate ligand and/or protonation of acidic residues in the D-de loop region (e.g., D1-Glu244) could occur, it might be still possible that the carbonate state is stabilized in the presence of Fe\(_{\text{2+}}\).

**Fig. S1.** Overview of typical potential-energy profiles: (Top) standard H-bonds (asymmetric double-well), typically with an $O_{\text{donor}}$–$O_{\text{acceptor}}$ distance $\sim 2.6$ Å; (Middle) low barrier H-bond (LBHB), typically with an $O_{\text{donor}}$–$O_{\text{acceptor}}$ distance of 2.5–2.6 Å; (Bottom) single-well (ionic) H-bonds, typically with an $O_{\text{donor}}$–$O_{\text{acceptor}}$ distance of $\sim 2.5$ Å (1). The corresponding O–N distances are generally greater than O–O distances.


**Fig. S2.** Potential-energy profiles of the H-bond between D1-His215 and the proximal $Q_a$ carbonyl in the presence of bicarbonate $\text{HCO}_3^-$ (red curve), formate (green curve), and carbonate $\text{CO}_3^{2-}$ (pink curve) ligands. At each point, all of the atomic coordinates in the QM region were fully relaxed (i.e., not fixed). Arrows indicate the directions of PT.
Table S1. Optimized H-bond distances in model complexes [His and trimethyl quinone (Q)] in vacuum (in Å)

<table>
<thead>
<tr>
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<th>Q</th>
<th>Q*</th>
<th>QH*</th>
<th>QH-</th>
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<tr>
<td>OQ(prox.)...H...NδHis</td>
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<td>2.71</td>
<td>2.95</td>
<td>2.62</td>
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<tr>
<td>OQ(prox.)...H</td>
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<td>1.93</td>
<td>1.54</td>
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<tr>
<td>H...NδHis</td>
<td>1.02</td>
<td>1.05</td>
<td>1.02</td>
<td>1.08</td>
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</table>

It should be noted that in a model complex in which the nonheme Fe was absent, the H-bond distance between His and Q was also shortest (2.62 Å) in the QH- state (with 2.71–2.95 Å found for other states). However, this distance is still longer than seen when the Fe is present; thus, the nonheme Fe complex also plays a role in decreasing the pκa of D1-His215 to match that of QBH- protonation, enabling the completion of QBH2 formation.

For atomic coordinates, see Dataset S1.

Table S2. Charge and spin density distribution in the [QA/QB] state in optimized geometries of QAFeQB complexes

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<th>Moiety</th>
<th>Charge</th>
<th>Spin</th>
<th>Charge</th>
<th>Spin</th>
<th>Charge</th>
<th>Spin</th>
<th>Charge</th>
<th>Spin</th>
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<td>-0.39</td>
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<td>0.22</td>
<td>0.08</td>
<td>0.22</td>
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<td>3.78</td>
<td>0.70</td>
<td>3.78</td>
</tr>
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<td>0.22</td>
<td>0.08</td>
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<td>0.08</td>
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*Table S3. Charge and spin density distribution in optimized geometries of QAFeQB complexes with the carbonate (CO3²-) ligand. The presence of CO3²- forces Fe²⁺ to release of an electron from to one of the quinones

<table>
<thead>
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<th>Moiety</th>
<th>Charge</th>
<th>Spin</th>
<th>Charge</th>
<th>Spin</th>
<th>Charge</th>
<th>Spin</th>
<th>Charge</th>
<th>Spin</th>
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<td>0.99</td>
<td>-0.77</td>
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<td>0.82</td>
<td>3.01</td>
<td>0.77</td>
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*Other Supporting Information Files

Dataset S1 (PDB)