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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 plastiquinone (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 QBH2. QBH2 exchanges with PQ from the pool in the membrane. Based on experimental evidence, the photosynthetic reaction centers of purple bacteria, which are thought to share a common ancestor with PSI and PSII, are equivalent to Ser-L223 and Asp-L213, respectively, in the reaction center of 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 to enter the medium from the reaction center to reach the QB reduction site. The residues involved in the proton transfer (PT) pathway include Glu-L212, Asp-L213, Ser-L223, and several residues of the H-subunit. For PSI, the current state of knowledge lacks these details, but several of the reactions are considered to be similar (reviewed in ref. 6, 19).

Here, we investigated formation of QBH+ and QBH2 in the PSI protein environment, 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 QB− to QBH+ via D1-His215 and D1-His252. To elucidate how the conversion of QB− to QBH+ occurs, we analyzed the potential-energy profiles of the two

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H-bonds of $Q_B^{+}$, $O_{QB}^{(distal)}\ldots H\ldots O_{D1-Ser264}$ and $O_{QB}^{(proximal)}\ldots H\ldots N_{D1-His215}$. Because in previous electrostatic calculations, D1-His252 became protonated upon formation of the $Q_B^{+}$ state (8), we also assumed the presence of protonated D1-His252 and the $Q_B^{+}$ state in the present QM/MM calculation. In general, serine is unlikely to deprotonate. However, in the $Q_B^{+}$ state, the potential-energy profile indicates that a PT from D1-Ser264 to $O_{QB}^{(distal)}$ occurred very easily in an energetically downhill process (Fig. 2). This reaction was accompanied by a concerted PT from protonated D1-His252 to $O_{QB}^{(distal)}$, resulting in the formation of $O_{B}^{(distal)}{^+}$, 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, $O_{ QB}^{(distal)} \ldots H \ldots O_{D1-Ser264}$ (2.48 Å) and $O_{D1-Ser264} \ldots H \ldots N_{D1-His252}$ (2.51 Å), are unusually short, especially in the $Q_B^{+}$ 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 $O_{QB}^{(proximal)} \ldots H\ldots N_{D1-His215}$ (Fig. 2) resembles that of a standard asymmetric double-well H-bond (22) (Fig. S1), suggesting that the first PT from D1-His215 to $O_{QB}^{(proximal)}$ is an energetically uphill process. This is primarily because proton release from the singly protonated (neutral) His ($pK_a \sim 14$ for imidazole) (23) is unfavorable, unlike the doubly protonated (positively charged) His, for which the $pK_a \sim 7$. Although the $pK_a$ 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 $Q_B^{+}$ is present compared with the neutral $Q_B$ state. This is similar to the situation occurring in the purple bacterial RC, where $Q_B^{+}$ is unprotonated on the first flash and the $Q_B^{+}$ to $Q_BH^+$ step only occurs when the $[Q_B^{+}\ldots Q_B^{+}]$ state is formed, before the second electron transfer step (15, 16).

Overall, the results suggest the following model for the first protonation: Protonation of $Q_B^{+}$ to $Q_BH^+$ primarily occurs at $O_{ QB}^{(distal)}$, this occurs as a result of concerted PT from protonated D1-His252 to D1-Ser264, and this induces reorientation of the D1-Ser264 hydroxyl group so that it can act as a H-bond acceptor from the $Q_BH^+$. The preferential occurrence of the first protonation at $O_{ QB}^{(distal)}$ over $O_{ QB}^{(proximal)}$ is consistent with the $Q_B$ protonation mechanism in purple bacterial RCs (15, 16, 24).

**Second PT and an Unusually Short H-Bond Distance Between $Q_BH^+$ and D1-His215.** Because $O_{QB}^{(distal)}$ is protonated upon $Q_BH^+$ formation (Fig. 2), the second protonation—i.e., the conversion of $Q_BH^+$ to $Q_BH_2^+$—must occur at $O_{QB}^{(proximal)}$, which is H-bonded by $N_6$ of D1-His215 (Fig. 1). The QM/MM-optimized H-bond distance between $O_{QB}^{(proximal)}$ of $Q_BH^+$ and $N_6$ of D1-His215...
was found to be unusually short (2.47 Å) in the QbH state (Table 1).

Intriguingly, this distance is identical to that in the 1.9 Å structural model, PSII monomer unit “A” of the PSI complexes (9). The corresponding O$_{QB(prox.)}$–H–N$_{D1-His215}$ distances were found to be 2.77 Å in the Qb state and 2.68 Å in the Qb$^{+*}$ state (Table 1), both being significantly longer compared with that in the QbH state. In addition, the corresponding O$_{QB(prox.)}$–H–N$_{D2-His214}$ distance (2.77 Å) should be treated with caution. Furthermore, the QbH state is expected to be a short-lived intermediate and not a state that would be present and in PSII (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 O$_{QB(prox.)}$–N$_{D1-His215}$ distance of 2.47 Å is an unusually short H-bond and may possess a single-well potential. Remarkably, the calculated potential-energy profiles for the O$_{QB(prox.)}$–N$_{D1-His215}$ H-bond in the QbH state resembled that of a barrierless single-well (ionic) H-bond, suggesting that the second PT can occur isenergetically at O$_{QB(prox.)}$ (Fig. 2).

The significantly elongated H–N bond of D1-His215 (1.15 Å) in the QbH state implies that further migration of an H atom toward the acceptor O$_{QB(prox.)}$ moiety (i.e., PT) can occur easily. Indeed, the single-well potential obtained for O$_{QB(prox.)}$–N$_{D1-His215}$ is symmetric (Fig. 2), implying that the pK$_a$ difference (27) between D1-His215 deprotonation and QbH protonation is close to zero.

The pK$_a$ for the OH$^–$ to OH$^+$ 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 pK$_a$ of 4.9 for the protonation of the semiquinone, Q$^{+*}$ to OH$^+$ (18, 28). The pK$_a$ 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 Fe$^{2+}$ should lower the pK$_a$ of neutral D1-His215. The pK$_a$ of the neutral His ligand to Fe$^{2+}$ in the Rieske (2Fe-2S) cluster has been measured to be ~12.5 rather than ~14 (29, 30). The ligand environment of the Fe$^{2+}$ in PSII is more positively charged than that in the Rieske cluster; thus, the pK$_a$ 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 pK$_a$ value of D1-His215 is likely to be close to that for the QbH$^–$ to OH$^+$ 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 QH$^+$ (15, 32, 33). This residue is 5.7 Å away from O$_{QB(prox.)}$ (34), and it is not clear how this last protonation reaction occurs. One possibility is that the QbH$^+$ 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 QbH$_2$ from the site. Such a scenario has been discussed (and disfavored) previously (15). Ionizable residues corresponding to Glu-L212 are absent near Qb 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 reprotontated via the bicarbonate ligand; indeed, a role for the bicarbonate in protonation of Qb has been considered for many years (21). The distance between O$_{QB(prox.)}$ and O$_{bicarbonate}$ 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 QbH$^+$, 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 Q$_{A}^{+*}$ and Q$_{B}^{+*}$. To do this, we redefined the QM region such that it included Q$_{A}$, the nonheme Fe and its ligands, Q$_{B}$, D1-Tyr246 and D2-Tyr244. Note that in the calculations, in the absence of the PSI 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* formation (characteristic δ of QA* was 0.20) was more pronounced and (|QA| = 0.50, [Fe(His)](HCO3)2−/QA| = 0.77, [QA]/|QB| = 0.22) 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 (QA*− favored orientation), QA* formation was high (|QA| = 0.06, [Fe(His)](HCO3)2−/QA| = 0.80, |QA|/|QB| = 0/84 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 QA*− and QB*− oriented conformations and the protonation state of the H-bond oxygen (40). We could not observe such a dramatic change of the Fe−aq state with the formate ligand, 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 PSII.

Recently it was suggested that the ligation of the Fe2+ by bicarbonate changes during electron transfer, giving from bidentate to monodentate upon formation of QA*− and returning to the bidentate form upon electron transfer to QB (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 bicarbonate and the quinone redox 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 biquinone 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 QB*−/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 QAH2 (39). The PT mechanisms reported here suggest that the efficient release of QAH2 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 the second protonation remains essentially unchanged and can occur isoenergetically at QAH2 (Fig. S2). The vibrational state O(H)−NO(D1-His215) distance was 2.49 Å in the QAH2 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+ QBH2 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 carbonate 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 QAH2 to QAH2 protonation process became slightly energetically uphill and the O(H)−NO(D1-His215) distance was increased by 2.4 Å in the QAH2 state (Table I 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 PSII protein environment with carbonate (QM region: QA, the nonheme Fe, the His and carbonate ligands, O6, D1-Tyr246, and D2-Tyr244). Starting with carbonate-ligated Fe2+ and two neutral quinones, the QM/MM calculations resulted in the oxidized Fe2+ a reduced quinone and a neutral quinone (Table S3). QM/MM calculations for the carbonate-ligated Fe2+, one reduced quinone, and one neutral quinone resulted in oxidized Fe3+ 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 PSII. 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 PSII 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-Å 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 PSII 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 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/D2-His215/OH* 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-His215/QBH2. 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 Chla, Phaeo, and quinones were taken from our previous studies on PSII (52). We considered the Mn2CaO5 cluster as the (O4)2-QBH2 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 Qtie (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 (QB, D1-His215, D1-His214, 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 (QB, D1-His252, D1-Ser264, bicarbonate, Fe, D1-His215, D1-His272, D2-His214, 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 (QB, D1-His252, D1-Ser264, bicarbonate, Fe, D1-His215, D1-His272, D2-His214, 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** and set the spin multiplicity of the system to S = 2 in calculations for Qh, Qh*, and Qh**, and S = 1/2 for Qs, Qs*, Qs**, and [Qs(Qh)]**. The geometries were redefined by constrained 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
after which the geometry was optimized by constraining the Odonor to the acceptor atom (Oacceptor) by 0.05 Å, after which the geometry was optimized by constraining the Odonor-H and O-acceptor distances, and the energy of the resulting geometry was calculated. This procedure was repeated until the H atom reached the Oacceptor atom.

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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 QB⁺ 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 QB⁺ 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 O₉B(proximal)–NδD₁-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⁺. Further reduction of QB⁺ 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 QB⁺ 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-δ loop region (e.g., D1-Glu244) could occur, it might be still possible that the carbonate state is stabilized in the presence of Fe²⁺.

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 Q₈ carbonyl in the presence of bicarbonate HCO$_3^-$ (red curve), formate (green curve), and carbonate 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>
<th>Q state</th>
<th>Q</th>
<th>Q±</th>
<th>QH⁺</th>
<th>QH⁻</th>
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</thead>
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<td>3.04</td>
<td>2.71</td>
<td>2.95</td>
<td>2.62</td>
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<tr>
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<td>1.93</td>
<td>1.54</td>
</tr>
<tr>
<td>H 0.02</td>
<td>1.02</td>
<td>1.05</td>
<td>1.02</td>
<td>1.08</td>
</tr>
</tbody>
</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 pKₐ of D1-His215 to match that of QBH⁻ protonation, enabling the completion of QBH₂ formation.

aFor atomic coordinates, see Dataset S1.

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

<table>
<thead>
<tr>
<th>Moiety</th>
<th>QA⁻</th>
<th>Charge</th>
<th>Spin</th>
<th>QA⁻ Fe²⁺</th>
<th>QB</th>
<th>Charge</th>
<th>Spin</th>
<th>QA⁻ Fe²⁺ QA⁻</th>
<th>QB</th>
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<th>Spin</th>
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aD1-His252 is an H-bond acceptor of D1-Ser264. D1-His252 is deprotonated (neutral). See Fig. 3.
bQB is an H-bond acceptor of D1-Ser264. D1-His252 is protonated (positively charged). See Fig. 3.
cNot included in the system.
dESP (electrostatic potential) charges.
eMullikan spin populations.
fD1-His215, D1-His272, D2-His214, D2-His268 and bicarbonate.

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

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<th>QA⁻</th>
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<th>Spin</th>
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<th>QB</th>
<th>Charge</th>
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Other Supporting Information Files

Dataset S1 (PDB)