Proton-mediated photoprotection mechanism in photosystem II

Yu Sugo1 and Hiroshi Ishikita1,2*

1Department of Applied Chemistry, The University of Tokyo, Tokyo, Japan, 2Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan

Photo-induced charge separation, which is terminated by electron transfer from the primary quinone QA to the secondary quinone QB, provides the driving force for O2 evolution in photosystem II (PSII). However, the backward charge recombination using the same electron-transfer pathway leads to the triplet chlorophyll formation, generating harmful singlet-oxygen species. Here, we investigated the molecular mechanism of proton-mediated QA•− stabilization. Quantum mechanical/molecular mechanical (QM/MM) calculations show that in response to the loss of the bicarbonate ligand, a low-barrier H-bond forms between D2-His214 and QA•−. The migration of the proton from D2-His214 toward QA•− stabilizes QA•−. The release of the bicarbonate ligand from the binding Fe2+ site is an energetically uphill process, whereas the bidentate-to-monodentate reorientation is almost isoenergetic. These suggest that the bicarbonate protonation and decomposition may be a basis of the mechanism of photoprotection via QA•−/QAH+ stabilization, increasing the QA redox potential and activating a charge-recombination pathway that does not generate the harmful singlet oxygen.

KEYWORDS
photoprotection, photoinhibition, low-barrier hydrogen bond (LBHB), bicarbonate, proton-coupled electron transfer (PCET), formate, D1-Tyr246, QBH2 release/exchange

Introduction

The driving force for photosynthetic O2 evolution is provided by the photoinduced charge separation at the reaction center of photosystem II (PSII). The electronic excitation of the chlorophyll leads to electron transfer via pheophytin in the D1 protein, PheoD1, to two plastoquinone molecules, QA and QB (Shen, 2015). The terminal electron acceptor QB accepts two electrons via the primary quinone QA and two protons. The non-heme Fe2+ complex, which comprises D1-His215, D2-His214, D1-His272, D2-His268, and bicarbonate (HCO3−), is equidistant from QA and QB (Shevela et al., 2012; Figure 1A). The bidentate bicarbonate is modeled in the majority of the reported PSII structures (e.g., Umena et al., 2011; Gisriel et al., 2022; Figure 1A), whereas not only the bidentate but also monodentate bicarbonate is modeled in the PsbM-deleted PSII
FIGURE 1
Overview of the quinone binding sites. (A) X-ray crystal structure of PSII from Thermosynechococcus vulcanus (PDB code, 3WU2) (Umena et al., 2011) and electron density map. (B) X-ray crystal structure of the PsbM-deleted PSII from Thermosynechococcus vestitus BP-1 (PDB code, 5H2F) (Uto et al., 2017) and electron density map. (C) Cryo-electron microscopy structure of PSII from Synechocystis sp. PCC 6803 (PDB code, 7N8O) (Ferreira et al., 2004). Dotted lines indicate H-bonds. The density of the D1-Tyr246 moiety, including the adjacent water molecule in the PsbM-deleted structure, is indicated by the red mesh. (D) Energetics of forward and backward electron-transfer processes. Processes occurring via the high-$E_m$ form are shown in red. P680* denotes the electronically excited P680.*

(Figure 1B; Uto et al., 2017). D1-Tyr246 and D2-Tyr244 are located at the bicarbonate binding site (Forsman et al., 2019). The characteristic of D1-Tyr246 is the electron density near the hydroxyl group (Saito et al., 2013), which is clearly observed in the 1.9-Å X-ray diffraction (XRD) (Umena et al., 2011) and X-ray free electron laser (XFEL)-S1 (Suga et al., 2015) structures (Figure 1A). The experimentally observed electron density was not interpreted in 2011 and 2015 (Figure 1A). In 2017, Uto et al. (2017) modeled the corresponding density as a water molecule (B-factor: 40 Å$^2$) forming a significantly short H-bond with the hydroxyl group of D1-Tyr246 (2.31 Å) (Figure 1B). Remarkably, the O$_{D1-Tyr246}$...O$_{water}$ distance of 2.3 Å is closer to the O...O distance for a typical Zundel (H$_2$O...H$^+$...H$_2$O) cation ($\approx$2.3 Å). The absence of the corresponding density at the D2-Tyr244 moiety may indicate a functional asymmetry between the Q$_A$ and Q$_B$ sides (Saito et al., 2013).

The redox potential ($E_m$) values of −100 to −140 mV for one-electron reduction of Q$_A$ (Q$_A$/Q$_A^-$) (Krieger and Weis, 1992; Johnson et al., 1995; Krieger et al., 1995; Ishikita and Knapp, 2005a; Shimamoto et al., 2009) and 90 mV for Q$_B$/Q$_B^-$...
E.*presence of Q₇B Synechocystis the formation of the high-

Reduced Qṃ exergonic electron transfer occurs under normal functional

(Kato et al., 2016; De Causmaecker et al., 2019) indicate that exergonic electron transfer occurs under normal functional conditions. Reduced Q₈ accepts the first proton via D1-Ser264 and D1-His252 at the distal carbonyl O site with respect to the non-heme Fe²⁺ complex (Ishikita and Knapp, 2005a; Saito et al., 2013; Ashizawa and Noguchi, 2014) and the second proton via D1-His215 at the proximal carbonyl O site (Saito et al., 2013, 2020; Kimura et al., 2020); thus, reduced Q₈ forms Q₈H₂ and moves from PSII toward the quinone pool. The depletion of the bicarbonate from the non-heme Fe²⁺ reduces the rate of the electron transfer from Q₈ to Q₇B (Jursinic et al., 1976; Eaton-Rye and Govindjee, 1988) or the exchange of Q₈H₂ (Siggel et al., 1977; Sedoud et al., 2011).

Under strong light, the plastoquinone pool is fully reduced, and the Q₈ binding site is unoccupied, which inhibits electron transfer and causes Q₇B to accumulate in PSII (photo-inhibition) (Vass et al., 1992; Noguchi, 2002). If the Eₘ₈ gap between Q₇A and PheoD₁ is small, backward electron transfer occurs in the form of charge recombination from Q₇B via PheoD₁ to the cationic chlorophyll in the reaction center, forming triplet chlorophyll and generating harmful singlet-oxygen species (Rutherford and Krieger-Liszkay, 2001; Rutherford et al., 2012). Q₇A has been reported to exhibit two forms, a low- and a high-Eₘ₈(Q₇A) conformation (Figure 1D; Krieger and Weis, 1992; Johnson et al., 1995; Krieger et al., 1995). Q₇A exists in the low-potential form under normal functional conditions. The high-Eₘ₈(Q₇A) conformation can increase the Eₘ₈ gap between Q₇A and PheoD₁, preventing charge recombination via PheoD₁ and singlet-oxygen generation under strong light [photoprotection (Rutherford and Krieger-Liszkay, 2001; Rutherford et al., 2012)].

The molecular origin of the high-Eₘ₈(Q₇A) form had long remained unsolved. Vass et al. (1992) proposed that the Q₇B stabilization is mediated by protonation. In the 3.5-Å crystal structure of PSII (Ferreira et al., 2004), the OH group of D2-Thr217 forms an H-bond with Q₇B but not with unprotonated neutral Q₇A (Ishikita and Knapp, 2005a). Thus, H-bond donation of D2-Thr217 to Q₇B could be a possible mechanism for the formation of the high-Eₘ₈ conformation. However, the corresponding H-bond is unlikely to form in the crystal structure of PSII from Thermosynechococcus vulcanus (Umena et al., 2011) and the recent cryo-electron microscopy structure of PSII from Synechocystis sp. PCC 6803 (Gisriel et al., 2022). The aforementioned crystal structure shows that Q₇A has D2-His214 at the proximal carbonyl O site and the backbone amide group of D2-Phe261 at the distal carbonyl O site as H-bond partners.

The non-heme Fe²⁺ complex might be involved in the photoprotection mechanism (Diner and Petrouelas, 1987; Muh et al., 2012). Notably, Brinkert et al. (2016) reported that the loss of the bicarbonate ligand from the non-heme Fe²⁺ complex leads to an increase of 75 mV in Eₘ₈(Q₇A) in the presence of Q₇A; this suggests that the loss of the bicarbonate ligand is responsible for the formation of the high-Eₘ₈(Q₇A) conformation. Recent studies have shown that photo-induced CO₂ conversion is likely to occur from the bicarbonate ligand at Fe²⁺ (Shevela et al., 2020). Bicarbonate loss and photo-induced CO₂ conversion likely constitute the basis of the photoprotection mechanism. Although the loss of the negatively charged bicarbonate (HCO₃⁻) certainly increases Eₘ₈(Q₇A), the distance between the bicarbonate ligand and Q₇A is more than 6 Å (Umena et al., 2011). Moreover, whether the proton-mediated Q₇B stabilization mechanism (Vass et al., 1992) is still relevant to the Q₇B stabilization remains unclear.

To understand the mechanism of how the loss of the bicarbonate increases Eₘ₈(Q₇A), we investigated the bicarbonate and Q₇B binding sites in PSII using a quantum mechanical/molecular mechanical (QM/MM) approach based on the PSII crystal structure (Umena et al., 2011).

Materials and methods

Coordinates and atomic partial charges

The atomic coordinates were obtained from the X-ray crystal structure of PSII (PDB code, 3WU2) (Umena et al., 2011). The heavy-atom positions were fixed while the H-atom positions were optimized with CHARMM (Brooks et al., 1983). All titratable groups were ionized if not otherwise specified. D1-His337 was considered to be protonated (Nakamura and Noguchi, 2017). Atomic partial charges were obtained from the CHARMM22 (MacKerell et al., 1998) parameter set for amino acids and previous studies for cofactors (Saito et al., 2015), respectively.

Quantum mechanical/molecular mechanical calculations

The unrestricted density functional theory method was employed with the B3LYP functional and LACVP* basis sets (LANL2DZ (double ζ quality basis set with the Los Alamos effective core potential) for Mn and Ca atoms and 6-31G* for other atoms) (Hay and Wadt, 1985) using the QSite (QSite, 2012) program. Counter ions were added to neutralize the system. In the QM region, all atomic coordinates were relaxed. In the MM region, the H-atom positions were energetically optimized, and the heavy-atom positions were fixed using the OPLS2005 force field because the MM region is used mainly to reproduce (long-distance) electrostatic interactions with the QM region and the heavy-atom positions in the MM region should remain unchanged with respect to those in the original crystal structure. The initial-guess wave functions were obtained using the ligand field theory (Vacque et al., 1999) implemented in the QSite program. Three QM regions were used: (i) [Q₇A, Q₈] the non-heme Fe complex (bicarbonate if applicable, Fe,
and side chains of D1-His215, D1-His272, D2-His214, and D2-His268), side chains of D1-His252, D1-Ser264, and D2-Phe261 (including backbone) for the analysis of the H-bond between QA and D2-His214; (ii) [QA, QB, the non-heme Fe complex (bicarbonate, Fe, and side chains of D1-His215, D1-His272, D2-His214, and D2-His268), side chains of D1-Tyr246, D1-Ser264, and D1-His252, and the modeled water molecule adjacent to D1-Tyr246] for the analysis of the proton transfer toward the bicarbonate ligand; and (iii) [QA, QB, the non-heme Fe complex (bicarbonate, Fe, and side chains of D1-His215, D1-His272, D2-His214, and D2-His268), side chains of D1-Tyr246, D1-Ser264, D1-His252, and protonated D1-Glu244, and water molecules in the H-bond network (water molecules A618 and A659 and the modeled water molecule adjacent to D1-Tyr246] for the analysis of the proton transfer from D1-Ser268 to the bicarbonate ligand.

All other protein units and cofactors were approximated by the MM force field (i.e., electrostatic influences are sufficiently considered in the MM region). Note that the residues in the proton transfer pathways must be included in the QM region to consider the formation/breakage of the covalent (H-)bonds during proton transfer. See Supplementary material for the atomic coordinates of the resulting QM region. As in a previous study (Chernev et al., 2011), the non-heme Fe complex was in a high-spin state of Fe^{2+}, and the spin multiplicity of the system was set to $S = 2$ in calculations for neutral QA and $S = 5/2$ for QA$^\text{−}$.

The QM/MM-optimized geometry was used as the initial geometry to analyze the potential energy profiles. For the analysis of the $\text{ND2-His214} \cdots \text{H}^+ \cdots \text{OQA}$ H-bond, the focusing H atom was moved along the N⋯O H-bond from 0.02 to 0.10 Å (e.g., 0.02 Å around the local energy minimum in some

![Figure 2](https://example.com/figure2.png)

**Figure 2**
QM/MM-optimized geometry and potential energy profile for the H-bond between QA and D2-His214 prior to electron transfer from QA to QB. (A) Neutral QA. (B) QA^\text{−}. (C) QA^\text{−} in the absence of the bicarbonate ligand. D1-Ser264 forms an H-bond with unprotonated D1-His252, as the electron is not transferred to QB (Ishikita and Knapp, 2005a). Note that the geometry of each intermediate conformation is fully QM/MM optimized.
cases), after which the geometry was optimized by constraining the N...H⁺ and H⁺...O distances and energy was calculated. For the analysis of the bidentate-to-monodentate reorientation of the bicarbonate ligand, the focusing 0.10 Å, after which the geometry was optimized by constraining the C[HCO3]...Fe distance and energy was calculated. For the analysis of the release of the bicarbonate ligand from the Fe site, the C[HCO3]...Fe distance was increased by 0.10 Å, after which the geometry was optimized by constraining the O[acceptor]...H distance and energy was calculated. For the analysis of the proton transfer from H₂O⁺ to D1-Tyr246 and the bicarbonate ligand, the focusing H atom was moved away from the electron donor O site from 0.02 to 0.10 Å, after which the geometry was optimized by constraining the HCO₃⁻...H distance and energy was calculated. For the analysis of the proton transfer from H₂O⁺ adjacent to D1-Ser268 to the bicarbonate ligand, the focusing H atom was moved to the electron acceptor O site by 0.10 Å, after which the geometry was optimized by constraining the C[acceptor]...H distance and energy was calculated. For the analysis of the protonated bicarbonate decomposition to H₂O + CO₂, the H₂O...CO₂ distance was increased by 0.10 Å, after which the geometry was optimized by constraining the H₂O...CO₂ distance and the energy was calculated.

**Results**

**Formation of a low-barrier H-bond between D2-His214 and QA upon bicarbonate loss**

The H-bond distance of 2.78 Å between D2-His214 and QA in the PSII crystal structure (Umena et al., 2011; Figure 1A) is closest to 2.76 Å for neutral unprotonated QA in the QM/MM-optimized geometry (Figure 2A). This suggests that QA is neutral Qa in the PSII crystal structure. Although the H-bond is shortened to 2.65 Å when QA is reduced to QA−, the potential-energy profile indicates that the H⁺ is localized at the D2-His214 moiety (Figure 2B).

Remarkably, the D2-His214...QA− H-bond transforms into a low-barrier H-bond (2.50 Å) in response to the loss of the bicarbonate ligand (Figure 2C). In addition to the electrostatic contribution of the loss of the negative charge to an increase in $E_{\text{ind}}(Q_A)$, the migration of the D2-His214 proton toward QA− along the low-barrier H-bond (0.23 Å) stabilizes QA− significantly. In addition to the loss of a negative charge, this is likely a substantial reason for the 75 mV upshift in $E_{\text{ind}}(Q_A)$ upon the loss of the bicarbonate (Brinkert et al., 2016) because QA−...HCO₃⁻ is not short (6.8 Å).

The formation of the low-barrier H-bond between QA− and D2-His214 suggests that $k_p(Q_A−/Q_AH) \approx k_p(D2-His214-NH/N−)$ in the absence of the bicarbonate. The low-barrier H-bond formation is also observed between D1-His215 and QbH⁺ in PSII (Saito et al., 2013) and His-L190 and QaH⁺ in photosynthetic reaction centers from purple bacteria (PhRc) (Sugo et al., 2021) during the QbH₂ formation; this suggests that $k_p(QbH−/QbH₂) \approx k_p(D1-His215-NH/N−)$ in the presence of the bicarbonate. $k_p(D1-His215-NH/N−)$ and $k_p(D2-His214-NH/N−)$ for deprotonation of singly protonated to anionic histidine are likely higher [e.g., 13 in the Rieske cluster (Zu et al., 2003; Hsueh et al., 2010)] than $k_p$ for deprotonation of doubly protonated to singly protonated histidine [e.g., 2–9 in protein environments (Grimsley et al., 2009)]. As $k_p(QH−/QH₂) = 11$ for plastoquinone (Q) in water (Hasegawa et al., 2017), a slight decrease in $k_p(D2-His214-NH/N−)$ due to Fe²⁺ can lead to $k_p(Q_A−/Q_AH) \approx k_p(D2-His214-NH/N−)$.

In the low-barrier H-bond, the proton can move easily between the two H-bond moieties (Ishikita and Saito, 2014). In the low-barrier H-bond between TyrZ and D1-His190, the 0.35 Å migration of the proton toward TyrZ from D1-His190 can increase $E_{\text{ind}}(TyrZ)$ by 130 mV (Saito et al., 2020). From the analogy, the > 0.2 Å migration of the proton toward QA− along the low-barrier H-bond (Figure 2C) is the most likely origin of the observed increase of 75 mV in $E_{\text{ind}}(Q_A)$ (Brinkert et al., 2016). Thus, QA− can be stabilized irrespective of QA−...HCO₃⁻ = 6.8 Å.

**Energetics of the bicarbonate displacement from the non-heme Fe complex**

In QM/MM calculations, the release of the bicarbonate ligand from the binding Fe²⁺ site is an energetically uphill process (Figure 3A), which suggests that the electrostatic interaction between Fe²⁺ and HCO₃⁻ is not negligibly small as long as the negatively charged HCO₃⁻ state is stable at Fe²⁺.

In contrast, the bidentate-to-monodentate reorientation is slightly uphill in QA−...QA−, as the monodentate bicarbonate can accept an H-bond from D1-Tyr246 (Figures 3B,C). Note that QA− is stabilized when the hydroxyl group of D1-Tyr246 is oriented toward QA− (Saito et al., 2013). It has been proposed that bicarbonate serves as not only a donor but also a monodentate ligand (Hienwadel and Berthomieu, 1995; Chernev et al., 2011; Uto et al., 2017). In vacuum, the monodentate ligation may become pronounced specifically upon the formation of QA− (Chernev et al., 2011), because the reorientation of the free bicarbonate ligand occurs easily and is the only way to compensate for the electrostatic repulsion against QA−. The corresponding electrostatic repulsion is smaller (bicarbonate...QA− = 6.8 Å) than the interactions with D1-Glu244 (3.4 Å from bicarbonate), D1-Tyr246 (3.2 Å), and D2-Tyr244 (3.1 Å) in the PSII protein environment. The shape of the potential-energy profile remains essentially unaffected in response to changes in the QA redox state (Figures 3B,C). Furthermore, the bidentate...
ligation is more stable than the monodentate ligation in the PSII protein environment (Figures 3B,C). Thus, the bidentate-to-monodentate reorientation is unlikely to synchronize with unimpaired electron transfer from QA to QB; the reorientation may occur only when the electron transfer is sufficiently slow to compete with the energetically uphill movement of the bicarbonate ligand.

These results suggest that the release of the bicarbonate ligand is energetically more uphill than the reorientation, as long as $\text{HCO}_3^-$ is ligated to the $\text{Fe}^{2+}$ site.

**Discussion**

**Possible mechanisms of bicarbonate loss**

The bicarbonate protonation and decomposition can also lead to the bicarbonate loss (e.g., Loerting et al., 2000). This requires the proton transfer pathway toward the bicarbonate binding site. Below we have discussed candidate proton-donor residues near the bicarbonate binding site.

Notably, the bicarbonate binding site is linked with the protein bulk surface via an H-bond network that is formed by D1-Glu244, D1-Tyr246, and D1-Ser264 (Figure 4). D1-Glu244 in the highly charged de-loop region was responsible for the pH dependence of $E_m$ for the non-heme Fe complex (Ishikita and Knapp, 2005b) and may be involved in the bicarbonate protonation at lower pH in the $\text{Fe}^{2+}$ state (Kato et al., 2021). In addition, the $E_m(\text{QA})$ shift was observed upon the D1-E244A mutation (Forsman et al., 2019). Indeed, D1-Glu244 is close to the bicarbonate (3.37 Å, Figure 1) (Umena et al., 2011). However, according to the geometry of the PSII crystal structure, ionized D1-Glu244 is stabilized by a salt bridge with D2-Lys264 (3.31 Å, Umena et al., 2011) and is unlikely to serve as a proton donor to the bicarbonate. Unless structural changes occur at higher pH, the geometry does not directly support the involvement of D1-Glu244 in the bicarbonate protonation.
FIGURE 4
Channel that proceeds from the bicarbonate binding site toward the stromal protein surface. (A) Overview of the hydrophobic channel (red mesh). (B) Cavity radius along the hydrophobic channel. The channel space was analyzed using CAVER (Chovancova et al., 2012).

Although D1-Ser268 does not form an H-bond with the bicarbonate ligand (4.57 Å, Umena et al., 2011), the existence of a water molecule, which is only 2.44 Å away from the D1-Ser268 side chain (2.44 Å, Umena et al., 2011), is remarkable. The short distance might be due to the water molecule being H$_3$O$^+$ [e.g., O...O = 2.4 Å for typical H$_2$O...H$_3$O$^+$ (Mikenda, 1986; Limbach et al., 2009; Figure 1A)]. However, assuming that the water molecule is H$_3$O$^+$ in the QM/MM calculation, H$_3$O$^+$ forms H-bonds with D1-Tyr246 and a water molecule adjacent to D1-Glu244, but not with D1-Ser268; this increases the $O_{D1−Ser268}...O_{H3O^+}$ distance to 2.98 Å (Figure 5). Furthermore, H$_3$O$^+$ is unstable at this site, releasing the proton to ionized D1-Glu244. H$_2$O$^+$ can exist at the D1-Ser268 moiety only when D1-Glu244 is protonated. However, proton transfer from H$_3$O$^+$ at the D1-Ser268 to the bicarbonate ligand is energetically uphill (Figure 5), which suggests that D1-Ser268 is unlikely the proton donor to the bicarbonate. D1-Ser268 and the adjacent water molecule may be more likely to be involved in the proton transfer associated with the Q$_B$H$_2$ release, as suggested for mutant PSII proteins (Forsman and Eaton-Rye, 2020).

D1-Tyr246 does not form an H-bond with the bicarbonate –OH group (3.80 Å) in the PSII crystal structure (Umena et al., 2011), whereas D1-Tyr246 forms an H-bond with the bicarbonate –OH group ($\approx$2.7 Å) in response to the bidentate-to-monodentate reorientation according to QM/MM calculations (Figure 3). D1-Tyr246 is located at the unique position, the interface between the channel that proceeds from the bicarbonate binding site (Figure 4) and the inner cavity, the Q$_B$ pocket.

The elongation electron density near the hydroxyl group of D1-Tyr246 (Saito et al., 2013) may be interpreted as a peroxide O with the O–O distance of 1.48 Å, which can be fitted to the density (Figure 1A; Saito et al., 2013). Although the peroxide O is oriented toward the Q$_B$ binding pocket, the link between the bicarbonate binding site and the Q$_B$ binding pocket via D1-Tyr246 is weak due to the less polar O site.

Alternatively, the elongation of the density may be interpreted as H$_3$O$^+$. In the crystal structure reported by Uto et al. (2017), the $O_{D1−Tyr246}...O_{water}$ distance of 2.3 Å is closer to the O...O distance for a typical Zundel (H$_2$O...H$^+$...H$_2$O) cation ($\approx$2.3 Å), which suggests that the density may originate from H$_3$O$^+$ (Figure 1B). In this case, the H$^+$ needs to enter via the Q$_B$-exchange channel, possibly during the Q$_B$ exchange.
FIGURE 6
Potential energy profile for proton transfer in the Q_B binding pocket, adding water molecules (*: H_2O-1 to 5). Note that the geometry of each intermediate conformation is fully QM/MM optimized.

FIGURE 7
H-bond network via D1-Tyr246 to the monodentate bicarbonate ligand. (A) QM/MM-optimized geometry before proton transfer. (B) Potential energy profile for proton transfer along the H_2O-...D1-Tyr246...bicarbonate H-bond network. (C) QM/MM-optimized geometry after proton transfer. (D) Potential energy profile for CO_2 release from the monodentate binding site. (E) QM/MM-optimized geometry after decomposition to H_2O and CO_2. Blue arrows indicate the orientations of the proton transfer. Red arrows indicate the orientations of the structural changes. Note that the geometry of each intermediate conformation is fully QM/MM optimized.
To investigate whether the uptake and existence of the H\(^+\) are energetically possible in the Q\(_B\) binding pocket, QM/MM calculations were performed, removing Q\(_B\) and filling the pocket with water molecules. The potential-energy profile indicates that the proton is energetically stable and populated among the two water molecules that bridge between D1-His215 and D1-Tyr246 (i.e., H\(_2\)O-4* and H\(_2\)O-5*), as H\(_2\)O\(^+\) is stabilized by three H-bond acceptor groups (Figure 6). After Q\(_B\) re-enters the binding pocket, the H\(^+\) is allowed to exist only at the non-excluded water molecules, e.g., the one adjacent to D1-Tyr246. Indeed, the bulky methoxy groups of ubiquinone in PbRC are replaced with the methyl groups of plastoquinone in PSII, allowing one water molecules to remain at the D1-Tyr246 moiety. Thus, a water molecule can remain at the D1-Tyr246 PSII, allowing one water molecules to remain at the D1-Tyr246 pocket. This is likely the cause of the elongation of the electron density adjacent to the -OH region of D1-Tyr246.

When H\(_2\)O\(^+\) is adjacent to D1-Tyr246, the bidentate-to-monodentate reorientation leads to the formation of an H-bond network that proceeds from H\(_2\)O\(^+\) via D1-Tyr246 toward the monodentate bicarbonate (Figure 7A). The potential energy profile for the H-bonds suggests that the H-bond network can serve as a proton-transfer pathway (Figure 7B).

Intriguingly, when the proton arrives at the bicarbonate moiety along the Grotthuss-like proton conduit, the bicarbonate undergoes protonation (Figure 7C) followed by conversion to H\(_2\)O and CO\(_2\) (Figure 7D). As the products move away from the Fe\(^{2+}\) site, the shape of O…C…O becomes linear, i.e., O – C = O (Figure 7E). Thus, the bicarbonate loss can occur through the conversion of the protonated bicarbonate to CO\(_2\) and H\(_2\)O at the Fe\(^{2+}\) moiety (Figure 7D), which is in line with the CO\(_2\) release observed on the electron-acceptor side (Shevela et al., 2020).

The bidentate-to-monodentate reorientation of the bicarbonate ligand is a low-barrier process (Figure 3). In the monodentate bicarbonate ligand, the H\(_2\)O\(^+\)...D1-Tyr246...HCO\(_3\)^–/Q\(_A\)^– H-bond network is formed. The hydroxyl group forms a Grotthuss-like proton transfer pathway, accepting the proton from H\(_2\)O\(^+\) and donating it to the bicarbonate. That is, D1-Tyr246 remains protonated during proton transfer, as suggested using Fourier transform infrared (FTIR) spectroscopy (Takahashi et al., 2009). The absence of the proton donor (H\(_3\)O\(^+\)), proton-conducting side chain (D1-Tyr246), and decomposable carboxylic ligand (bicarbonate) in PbRC implies that the proton-mediated Q\(_A\)^– stabilization occurs specifically in O\(_2\)-evolving PSII for photoprotection. The involvement of D1-Tyr246 in the photoprotective role (proton-mediated Q\(_A\)^– stabilization) is in line with the findings of mutational studies; the photosautotrophic growth was slower in the D1-Y246F mutant PSII than in wild-type PSII (Kless et al., 1994); the D1-Y246A mutant PSII was also unable to grow photoautotrophically under strong light (Forsman et al., 2019).

The polar –OH region of D1-Tyr246 on the surface of the Q\(_B\) binding pocket can mediate the conduction of the proton to the bicarbonate binding site. The low-barrier bidentate-to-monodentate reorientation contributes to the formation of the “proton wire” (Stuchebrukhov, 2009) between D1-Tyr246 and the unligated –OH site of the bicarbonate. The corresponding H-bond formation with D1-Tyr246 does not occur upon the replacement of bicarbonate with formate, because formate has no –OH site. The absence of the –OH site may also be an origin of why formate substitution inhibits electron transfer from Q\(_A\) to Q\(_B\) (Eaton-Rye and Govindjee, 1988) or the exchange of Q\(_B\)H\(_2\) (Sedoud et al., 2011). Thus, the hydrophobic and pre-organized protein electrostatic environment (Warshel, 1998; Warshel et al., 2006) on the bicarbonate binding site is likely to reduce the energy barrier for the bicarbonate protonation with respect to the bulk region, facilitating the direct protonation of the –OH site of the bicarbonate and the release of the product, gaseous CO\(_2\), without the formation of the typical (HO)\(_2\)C = O intermediate (Loerting et al., 2000).
Molecular dynamics simulations suggested that the polar –OH site of the bicarbonate is linked to water molecules in the Qb binding pocket via D1-Tyr246 during QbH2 release (Sugo et al., 2022). These water molecules serve as a proton transfer pathway for the reprotonation of D1-His215 (Sugo et al., 2022), the proton donor during QbH−/QbH2 conversion (Saito et al., 2013, 2020). Thus, the link between the polar –OH site and D1-Tyr246 may provide a key to understand how the bicarbonate is associated with QbH2 release, interacting with water molecules in the reprotonation pathway for D1-His215.

Hydrophobic residues that form a CO2-releasing channel

QM/MM calculations suggest that the product, CO2, enters the channel that proceeds via D1-Glu244 and D1-Tyr246 toward the stromal protein surface (Figure 4). The entrance of the channel is hydrophobic, being formed by the side chains of D1-Tyr246, D2-Phe235, and D1-Ile248. D1-Tyr246 may play two roles in the protonation of bicarbonate. While the polar –OH region mediates the conduction of the proton to the bicarbonate protonation site, the hydrophobic ring region can isolate the bicarbonate from the water molecules in the Qb binding pocket, making the bicarbonate binding moiety hydrophobic. The loss of the solvation also increases pK4(HCO3−/H2CO3) with respect to the bulk water region, facilitating the conversion of bicarbonate to gaseous CO2.

Conclusion

In response to the loss of the bicarbonate ligand, a short low-barrier H-bond forms between D2-His214 and QA−, which facilitates the proton migration toward QA− and increases $E_m(QA)$ (Figure 2). The loss of bicarbonate may be induced by the protonation. The D1-Glu244 conformation identified in the PSII crystal structure does not support the role in donating a proton to the bicarbonate ligand, whereas D1-Tyr246 may facilitate bicarbonate protonation and decomposition into H2O and CO2 (Figure 7). The channel, which is formed by D1-Tyr246, D2-Phe235, and D1-Ile248, may serve as a CO2-releasing channel (Figure 4). These constitute the basis for the photoprotection mechanism through the high-$E_m(QA)$ conformation, which inhibits the backward electron transfer from QA− via the triplet-generating pheophytin/chlorophyll cofactors (Figure 7). The proton-mediated photoprotection mechanism is pronounced exclusively in PSII, as Glu-M234 exists permanently and QA never leaves in PbRC (Wei et al., 2022). These findings could elucidate how the type-II reaction centers show notable structural differences not only on the luminal oxygen-evolving side but also on the stromal electron acceptor side.

Data availability statement

The original contributions presented in this study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HI designed the research and wrote the manuscript. YS and HI performed the research and analyzed the data. Both authors contributed to the article and approved the submitted version.

Funding

This research was supported by JST CREST (JPMJCR1656 to HI), JSPS KAKENHI (JP18H01937, JP18H05155, JP20H03217, and JP20H05090 to HI), and Interdisciplinary Computational Science Program in CCS, University of Tsukuba.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.934736/full#supplementary-material
Ashizawa, R., and Noguchi, T. (2014). Effects of hydrogen bonding interactions on the redox potential and molecular vibrations of plastoquinone as studied using density functional theory calculations. Phys. Chem. Chem. Phys. 16, 11864–11876. doi: 10.1039/c4cp04742e

Brinkert, K., De Causmaecker, S., Krieger-Liszkay, A., Fantuzzi, A., and Rutherford, A. W. (2016). Bicarbonate-induced redox tuning in Photosystem II for regulation and protection. Proc. Natl. Acad. Sci. U.S.A. 113, 12144–12149. doi: 10.1073/pnas.1608862113

Brooks, B. R., Brucoleri, R. E., Olafson, B. D., States, D. J., Swaminathan, S., and Karplus, M. (1983). CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. J. Comput. Chem. 4, 187–217. doi: 10.1002/jcc.540040211

Chernov, N., Zhang, W., Dau, H., and Haumann, M. (2011). Carboxylate shifts steer interquinone electron transfer in photosynthesis. J. Biol. Chem. 286, 5366–5374. doi: 10.1074/jbc.M110.228709

Chovancova, E., Pavelka, A., Benes, P., Strnad, O., Brezovsky, J., Kozlikova, B., et al. (2012). CAVER 3.0: a tool for the analysis of transport pathways in dynamic protein structures. PLoS Comput. Biol. 8, e1002708. doi: 10.1371/journal. pcbi.1002708

De Causmaecker, S., Douglass, J. S., Fantuzzi, A., Nitschke, W., and Rutherford, A. W. (2019). Energetics of the exchangeable quinone Q in photosystem II. Proc. Natl. Acad. Sci. U.S.A. 116, 19548–19546. doi: 10.1073/pnas.191075116

Diner, B. A., and Petrouleas, V. (1987). Qm, the non-heme iron of the photosystem II iron-quinone complex. A spectroscopic probe of quinone and inhibitor binding to the reaction center. Biochim. Biophys. Acta 895, 107–125. doi: 10.1016/S0006-3002(87)80010-1

Eaton-Rye, J. J., and Govindjee. (1988). Electron transfer through the quinone acceptor complex of photosystem II after one or two actinic flashes in bicarbonate-depleted spinach thylakoid membranes. Biochim. Biophys. Acta 935, 248–257. doi: 10.1016/0006-2788(88)90211-6

Ferreira, K. N., Iversen, T. M., Maglhaois, K., Barber, J., and Iwata, S. (2004). Architecture of the photosynthetic oxygen-evolving center. Science 303, 1831–1838. doi: 10.1126/science.1093087

Forsman, J. A., and Eaton-Rye, J. J. (2020). The D1:Ser268 residue of the D1 protein is involved in multiple quinone and herbicide interactions in photosystem II. Biochemistry 59, 19458–19463. doi: 10.1021/acs.biochem.1002398

Gisriel, C. J., Wang, J., Liu, J., Flesher, D. A., Reiss, K. M., Huang, H.-L., et al. (2014). Infrared determination of the protonation state of a key histidine ligand in the iron-quinone complex of photosystem II as revealed by low-level ATIR-FTIR spectroscopy. Biochemistry 59, 4336–4343. doi: 10.1021/acs.biochem.0c00810

Kimura, M., Kato, Y., and Noguchi, T. (2020). Protonation state of a key histidine ligand in the iron-quinone complex of photosystem II as revealed by low-level ATIR-FTIR spectroscopy. Biochemistry 59, 4336–4343. doi: 10.1021/acs.biochem.0c00810

Krieger, A., and Wei, E. (1992). Energy dependent quenching of chlorophyll fluorescence: the involvement of proton-calcium exchange at photosystem II. Photosynthetica 27, 89–98

Krieger, A., Rutherford, A. W., and Johnson, G. N. (1995). On the determination of redox midpoint potential of the primary quinone electron transfer acceptor, Qa, in photosystem II. Biochim. Biophys. Acta 1229, 193–201. doi: 10.1016/0006-2788(95)00002-Z

Limbach, H.-H., Tolstoy, P. M., Pérez-Hernández, N., Gao, J., Scherodivach, I. G., and Denisov, G. S. (2009). OHO hydrogen bond geometries and NMR chemical shifts: from equilibrium structures to geometric H/D isotope effects, with applications for water, protonated water, and compressed ice. Int. J. Chem. 49, 199–216. doi: 10.1560/IJCC.49.2.199

Loerting, T., Tautermann, C., Kromer, T. R., Kohl, I., Hallbrucker, A., Mayer, E., et al. (2000). On the surprising kinetic stability of carbonic acid (H2CO3). Angew. Chem. Int. Ed. 39, 891–894. doi: 10.1002/1521-3773(20000303)39:5<891::AID-anie891>3.0.CO;2-e

MacKerell, A. D., Jr., Bashford, D., Bellott, R. L., Jr., E, £, Mayer, A., et al. (2000). On the surprising kinetic stability of carbonic acid (H2CO3). Angew. Chem. Int. Ed. 39, 891–894. doi: 10.1002/1521-3773(20000303)39:5<891::AID-anie891>3.0.CO;2-e

Nakamura, S., and Noguchi, T. (2017). Infrared determination of the protonation state of a key histidine residue in the photosynthetic water oxidizing center. J. Am. Chem. Soc. 139, 9364–9375. doi: 10.1021/jacs.7b04924

Noguchi, T. (2002). Dual role of triplet localization on the accessory chlorophyll in the photosystem II reaction center: photoprotection and photodamage of the D1 protein. Plant Cell Physiol. 43, 1112–1116. doi: 10.1093/pcp/pcf137

QSite (2012). version 5.8. New York, NY: Schiringer LLC.

Rutherford, A. W., and Krieger-Liszkay, A. (2001). Herbicide-induced oxidative stress in photosystem II. Trends Biochem. Sci. 26, 648–653. doi: 10.1016/S0968-0004(01)01953-3

Rutherford, A. W., Oszczka, A., and Rappaport, F. (2012). Back-reactions, short-circuits, leaks and other energy wasteful reactions in biological electron transfer: redox tuning to survive life in O3. FEBS Lett. 586, 603–616. doi: 10.1016/j.febslet.2011.05.021

Sugio and Ishikita
Saito, K., Mandal, M., and Ishikita, H. (2020). Redox potentials along the redox-active low-barrier H-bonds in electron transfer pathways. Phys. Chem. Chem. Phys. 22, 25467–25473. doi: 10.1039/d0cp04265j

Saito, K., Rutherford, A. W., and Ishikita, H. (2013). Mechanism of proton-coupled quinone reduction in Photosystem II. Proc. Natl. Acad. Sci. U.S.A. 110, 954–959. doi: 10.1073/pnas.1212957110

Saito, K., Rutherford, A. W., and Ishikita, H. (2015). Energetics of proton release on the first oxidation step in the water-oxidizing enzyme. Nat. Commun. 6:8488. doi: 10.1038/ncomms9488

Sedoud, A., Kastner, L., Cox, N., El-Alaoui, S., Kirilovsky, D., and Rutherford, A. W. (2011). Effects of formate binding on the quinone-electron acceptor complex of photosystem II. Biochim. Biophys. Acta 1807, 216–226. doi: 10.1016/j.bbabio.2010.10.019

Shen, J. R. (2015). The structure of photosystem II and the mechanism of water oxidation in photosynthesis. Annu. Rev. Plant. Biol. 66, 23–48. doi: 10.1146/annurev-arplant-050312-120129

Shevela, D., Do, H.-N., Fantuzzi, A., Rutherford, A. W., and Messinger, J. (2020). Bicarbonate-mediated CO₂ formation on both sides of photosystem II. Biochemistry 59, 2442–2449. doi: 10.1021/acs.biochem.0c00208

Shevela, D., Eaton-Rye, J. J., Shen, J. R., and Govindjee. (2012). Photosystem II and the unique role of bicarbonate: a historical perspective. Biochim. Biophys. Acta 1817, 1134–1151. doi: 10.1016/j.bbabio.2012.04.003

Shibamoto, T., Kato, Y., Sugiura, M., and Watanabe, T. (2009). Redox potential of the primary plastquinone electron acceptor QA in photosystem II from Thermosynechococcus elongatus determined by spectroelectrochemistry. Biochemistry 48, 10682–10684. doi: 10.1021/bi901691j

Siggel, U., Khanna, R., Renger, G., and Govindjee. (1977). Investigation of the absorption changes of the plasto-quinone system in broken chloroplasts. The effect of bicarbonate-depletion. Biochim. Biophys. Acta 462, 196–207. doi: 10.1016/0005-2728(77)90202-x

Stuchebrukhov, A. A. (2009). Mechanisms of proton transfer in proteins: localized charge transfer versus delocalized soliton transfer. Phys. Rev. E Stat. Nonlin. Soft Matter Phys. 79:031927. doi: 10.1103/PhysRevE.79.031927

Suga, M., Akita, F., Hirata, K., Ueno, G., Murakami, H., Nakajima, Y., et al. (2015). Native structure of photosystem II at 1.95 Å resolution viewed by femtosecond X-ray pulses. Nature 517, 99–103. doi: 10.1038/nature13991

Sugo, Y., Saito, K., and Ishikita, H. (2021). Mechanism of the formation of proton transfer pathways in photosynthetic reaction centers. Proc. Natl. Acad. Sci. U.S.A. 118, e2103203118

Sugo, Y., Saito, K., and Ishikita, H. (2022). Conformational changes and H-bond rearrangements during quinone release in photosystem II. Biochemistry doi: 10.1021/acs.biochem.1022c00324

Takahashi, R., Boussac, A., Sugura, M., and Noguchi, T. (2009). Structural coupling of a tyrosine side chain with the non-heme iron center in photosystem II as revealed by light-induced Fourier transform infrared difference spectroscopy. Biochemistry 48, 8994–9001. doi: 10.1021/bi901195e

Umena, Y., Kawakami, K., Shen, J.-R., and Kamiya, N. (2011). Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature 473, 55–60.

Uto, S., Kawakami, K., Umena, Y., Iwai, M., Ikeuchi, M., Shen, J. R., et al. (2017). Mutual relationships between structural and functional changes in a PsbM-deletion mutant of photosystem II. Faraday Discuss 198, 107–120. doi: 10.1039/c6fd00213g

Vacek, G., Perry, J. K., and Langlois, J. M. (1999). Advanced initial-guess algorithm for self-consistent-field calculations on organometallic systems. Chem. Phys. Lett. 310, 189–194.

Vass, I., Stirling, S., Hundal, T., Koivuniemi, A., Aro, E. M., and Andersson, B. (1992). Reversible and irreversible intermediates during photoinhibition of photosystem II: stable reduced QA species promote chlorophyll triplet formation. Proc. Natl. Acad. Sci. U.S.A. 89, 1408–1412. doi: 10.1073/pnas.89.4.1408

Warshel, A. (1998). Electrostatic origin of the catalytic power of enzymes and the role of preorganized active sites. J. Biol. Chem. 273, 27035–27038. doi: 10.1074/jbc.273.42.27037

Warshel, A., Sharma, P. K., Kato, M., and Parson, W. W. (2006). Modeling electrostatic effects in proteins. Biochim. Biophys. Acta 1764, 1647–1676.

Wei, R. J., Zhang, Y., Mao, J., Kaur, D., Khaniya, U., and Gunner, M. R. (2022). Comparison of proton transfer paths to the QA and QB sites of the Rh. sphaeroides photosynthetic reaction centers. Photosynth. Res. doi: 10.1007/s11120-11022-00906-x

Zu, Y., Couture, M. M., Kolling, D. R., Crofts, A. R., Ellis, I. D., Fee, J. A., et al. (2003). Reduction potentials of Ruske clusters: importance of the coupling between oxidation state and histidine protonation state. Biochemistry 42, 12400–12408. doi: 10.1021/bi350957.