BBA - Bioenergetics 1860 (2019) 611–617

Contents lists available at ScienceDirect

BBA - Bioenergetics

journal homepage: www.elsevier.com/locate/bbabio

Bicarbonate rescues damaged proton-transfer pathway in photosystem II

Gourab Banerjee[a,1], Ipsita Ghosh[a,1], Christopher J. Kim[b], Richard J. Debus[b,*], Gary W. Brudvig[a,*]

[a] Department of Chemistry, Yale University, New Haven, CT 06520-8107, USA
[b] Department of Biochemistry, University of California, Riverside, CA 92521, USA

ARTICLE INFO

Keywords:
Bicarbonate
Oxygen evolution
Photosystem II
Proton transfer
Water oxidation

ABSTRACT

The membrane-protein complex photosystem II (PSII) catalyzes photosynthetic water oxidation. Proton transfer plays an integral role in the catalytic cycle of water oxidation by maintaining charge balance to regulate and ensure the efficiency of the process. The hydrogen-bonded amino-acid residues that surround the oxygen-evolving complex (OEC) provide an efficient pathway for proton removal. Hence, it is crucial to identify these pathways to provide deeper insights into the proton-transfer mechanisms. In this study, we have used bicarbonate as a mobile exogenous proton-transfer reagent to recover the activity lost by site-directed mutations in order to identify amino-acid residues participating in the proton-transfer pathway. We find that bicarbonate restores efficient S-state cycling in D2-K317A PSII core complexes, but not in D1-D61A and CP43-R357K PSII core complexes, indicating that bicarbonate chemical rescue can be used to differentiate single-point mutations affecting the pathways of proton transfer from mutations that affect other aspects of the water-oxidation mechanism.

1. Introduction

Photosynthetic water oxidation produces oxygen that is essential for the existence of aerobic organisms. The water-oxidation chemistry takes place in a 700 kDa pigment-protein complex called photosystem II (PSII). PSII is present in the thylakoid membranes of photosynthetic organisms including cyanobacteria, algae and green plants. It is comprised of a number of subunits, including the core proteins D1, D2, CP43 and CP47. The water-oxidation process is initiated by a photo-induced charge separation at P_{680}, which is a group of chlorophyll *a* molecules that function as the primary electron donor. The excited P_{680}* results in an electron transfer to a pheophytin molecule present on the electron acceptor side of PSII (Fig. 1). The acceptor side also contains two quinone electron acceptors, called Q_A and Q_B, a non-heme iron and a bicarbonate ion. Electrons are transferred from $Pheo^-$ to the primary quinone electron acceptor Q_A. The secondary quinone electron acceptor, Q_B, receives electrons from Q_A and forms plastoquinol, which diffuses out to the quinone pool in the thylakoid membrane. Simultaneously, the hole formed at P_{680} results in the oxidation of Tyr_Z, present on the donor side of the PSII. The donor side also contains the active site for catalysis of water oxidation, a Mn_4CaO_5 cluster called the oxygen-evolving complex (OEC) that is surrounded by a hydrogen-bonded network of waters and amino-acid residues [1–6].

The OEC cycles through a four-step process to form oxygen. The light-induced charge separation at P_{680} resulting in Tyr_Z oxidation triggers a one-electron oxidation of the Mn_4CaO_5 cluster in the OEC forming a series of intermediate oxidation states called S_i states, i = 0–4 [5,9,10]. The S-state cycling efficiency of the OEC is dependent on the choreographed removal of protons and electrons. The surrounding hydrogen-bonded network of amino-acid residues, two inorganic Cl^- ions and waters on the donor side (Fig. 2), as well as bicarbonate and non-heme iron on the acceptor side (Fig. 1), facilitate electron, proton and water transport, and help to maintain the redox potential of PSII, thereby ensuring an efficient water-oxidation process [11–15].

The crucial role of bicarbonate ion in PSII has long been known with the discovery of the “bicarbonate effect” [13,16–19]. Crystallographic studies along with membrane inlet mass spectrometry (MIMS) measurements have demonstrated the presence of a single bicarbonate ion bound to the non-heme iron on the acceptor side of the PSII [8,20–23]. EPR, spectroelectrochemical and computational studies have revealed that this bicarbonate assists in the second protonation step of Q_B reduction [24–27]. In addition, there have been proposals of an additional role of bicarbonate in the water-oxidation mechanism [28]. There are contrasting proposals regarding the binding sites and the function of bicarbonate ions on the donor side of the PSII [13,29]. Studies of the photoactivated assembly of the OEC support a role of a

* Corresponding authors.
E-mail addresses: richard.debus@ucr.edu (R.J. Debus), gary.brudvig@yale.edu (G.W. Brudvig).
[1] These authors contributed equally.

https://doi.org/10.1016/j.bbabio.2019.06.014
Received 24 April 2019; Received in revised form 18 June 2019; Accepted 21 June 2019
Available online 24 June 2019
0005-2728/

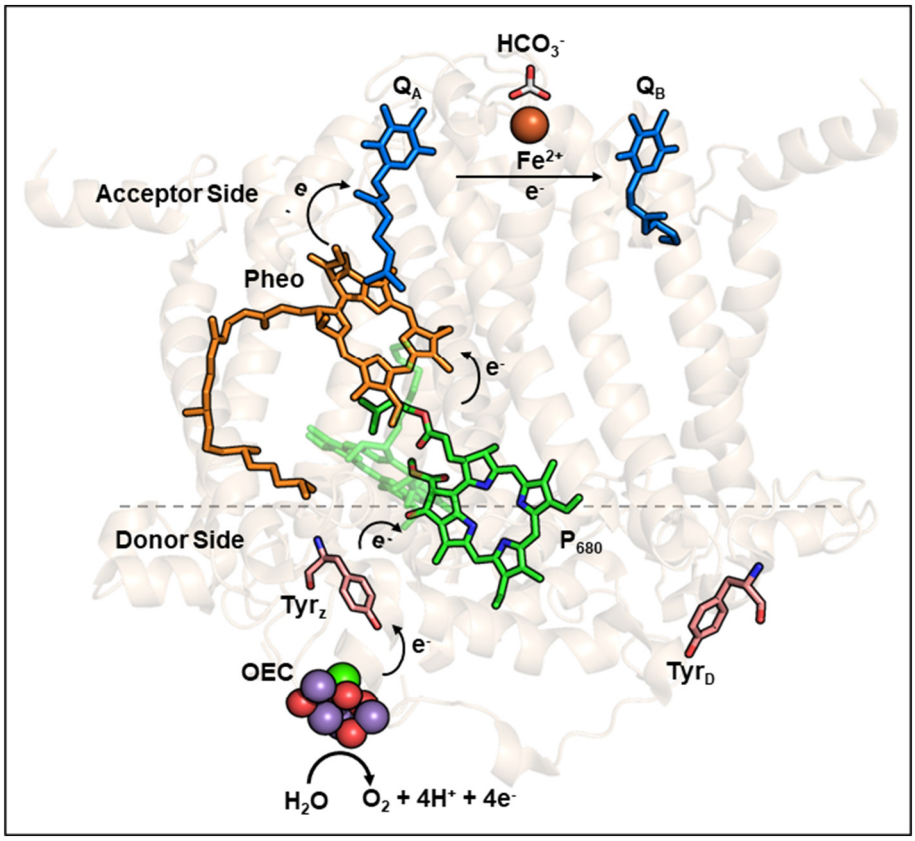

Fig. 1. The donor and acceptor sides of photosystem II. The donor side contains the Mn_4CaO_5 complex (OEC), where the Mn ions are marked in purple, O in red and Ca in green, Tyr_z (a tyrosine molecule in the D1 subunit of PSII, D1-Y161) and $P_{680,}$ which is a group of chlorophyll *a* molecules that function as the primary electron donor. The acceptor side contains a pheophytin molecule (Pheo) that accepts electrons from $P_{680.}$ The electrons are transferred from $Pheo^-$ to the primary quinone electron acceptor Q_A and then to the secondary quinone electron acceptor, Q_B. The Fe^{2+} and HCO_3^- ions on the acceptor side assist in electron transfer from Q_A to Q_B. Tyr_D (a tyrosine molecule in the D2 subunit of PSII, D2-Y160) plays an important role in the oxidation of the S_0 to the S_1 state [7]. The residues are marked in distinct colors for clarity and follow from the 3WU2 structure [8].

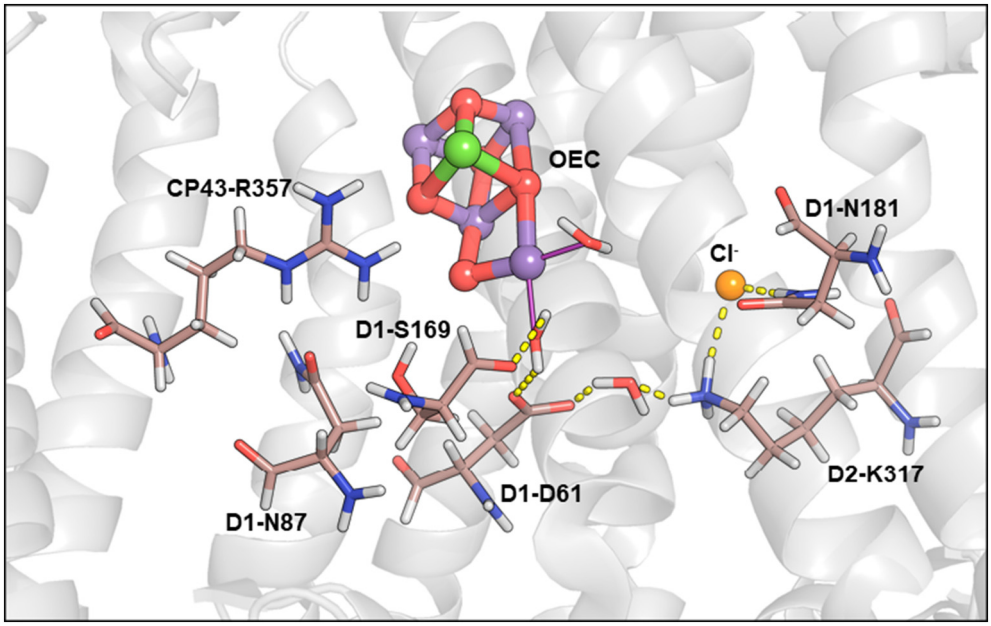

Fig. 2. The hydrogen-bonding network around the OEC depicting key amino-acid residues, chloride and waters. The C atoms are marked in pink, O atoms in red, Mn in purple, N in blue, Ca in green and Cl^- in orange. The dotted lines depict the hydrogen-bonding interactions. The figure follows from the 3WU2 structure [8].

bicarbonate as a ligand to the Mn(II) ions involved in the assembly process [30,31]. It has also been argued that bicarbonate plays a crucial role in stabilizing the OEC or can act as a substrate in water oxidation [16,32,33]. There is substantial evidence for a role of bicarbonate as a proton acceptor [21,34]. However, crystallographic studies, FT-IR spectroscopy and mass spectrometric studies have clearly demonstrated the absence of a bound bicarbonate ion on the donor side of PSII [35–38]. Further, MIMS studies have identified waters as substrates in the water-oxidation mechanism [39]. Hence, we can exclude the possibility of bicarbonate functioning as a ligand to the OEC or a substrate in the oxygen-evolution reaction.

In this study, we utilize bicarbonate as a mobile proton carrier to probe the proton-transfer pathway on the donor side of PSII. Analysis of the single-point mutations D1-D61A, D2-K317A, D1-E65A, D1-R334A using FT-IR studies and flash-induced polarographic measurements has been instrumental in tracing the proton-transfer pathway [40–42].

Computational analyses have also designated these residues as a part of the proton-transfer channel (Fig. 2) [43]. However, it is important to design experiments that will directly probe the proton-transfer reaction. Previously, chemical-rescue agents and genetic modulations have been used to understand the proton-transfer pathways in other enzymes including cytochrome *c* oxidase, carbonic anhydrase and heme-containing proteins [44–46]. In bacterial photosynthetic reaction centers, revertant mutations have been used to recover proton transfer to Q_B [47–49]. Further, imidazole and neutral weak acids have been used to restore proton transfer following mutations on the acceptor side of bacterial reaction centers [50–52]. Bicarbonate can also act as an exogenous proton carrier (reaction 1). It has previously been used to recover the activity of PSII in the presence of inhibitory zwitterions [53]. In this study, we have used bicarbonate as a mobile exogenous proton-transfer reagent to recover the function lost by site-directed mutations in order to identify amino-acid residues participating in the proton-transfer pathway. We find that bicarbonate restores efficient S-state cycling in D2-K317A PSII core complexes, but not in D1-D61A and CP43-R357K PSII core complexes.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \overset{\underset{6.37}{pk_a}}{\rightleftharpoons} H^+ + HCO_3^- \overset{\underset{10.25}{pk_a}}{\rightleftharpoons} H^+ + CO_3^{2-} \quad (1)$$

2. Materials and methods

2.1. Isolation of PSII from wild type and mutant strains of Synechocystis PCC 6803

The His-tagged wild type cells, and the site-directed mutations D1-D61A, D2-K317A, CP43-R357K and D1-N87A, were constructed as described previously [40,41,54,55]. Single colonies were selected for their ability to grow on solid media containing 5 μg/mL kanamycin monosulfate and 20 μg/mL gentamycin sulfate [56]. The cells were propagated in three 10 L carboys as described previously [54] and bubbled with 1% CO_2 in air. The PSII extraction and purification were done under dim light conditions at 4 °C using a Ni-NTA super flow affinity resin (Qiagen, Valencia, CA) as described previously [57]. The purified PSII core complexes were concentrated to ~ 1 mg of Chl/mL and stored in a buffer solution containing 1.2 M betaine, 10% (*v*/v) glycerol, 50 mM MES-NaOH (pH 6.0), 20 mM $CaCl_2$, 5 mM $MgCl_2$, 50 mM histidine, 1 mM EDTA, and 0.03% (*w*/*v*) *n*-dodecyl β-D-maltoside at −80 °C.

2.2. Steady-state oxygen assay

Oxygen evolution was monitored with a Clark-type electrode, and the oxygen-assay chamber was maintained at 25 °C using a temperature-controlled water bath. Samples were illuminated with an Oriel 1000 W tungsten–halogen lamp. 250 μM PPBQ with 1 mM K_3FeCN_6 was used as the electron acceptor and 5 μg of Chl was used in each assay.

To study the effect of bicarbonate, PSII core complexes isolated from wild type, D2-K317A, D1-D61A, CP43-R357K and D1-N87A *Synechocystis* cells were stirred in a buffer containing 8.4 mM sodium bicarbonate (different concentrations of bicarbonate were used for assay of the concentration dependence), 1 M sucrose, 60 mM Cl^-, 5 mM Ca^{2+} and 50 mM MES-NaOH (pH 6.5) in the dark for 2 min prior to each oxygen-evolution assay. This assay was compared to the oxygen-evolution assay in a buffer containing 1 M sucrose, 60 mM Cl^-, 5 mM Ca^{2+} and 50 mM MES-NaOH (pH 6.5).

2.3. Polarographic oxygen measurements

The flash-induced O_2 yields were measured polarographically by using a bare platinum electrode poised at −700 mV with a silver counter electrode. An EG&G Xe flash lamp controlled by an Arduino Uno (Ivrea, Italy) board provided periodic flashes interspersed by a delay time of 1 s. The measurements were performed in a buffer containing 1 M sucrose, 10 mM $CaCl_2$, 200 mM NaCl, and 50 mM MES-NaOH (pH 6.50) as described previously [58]. To study the effect of bicarbonate, the PSII core complexes isolated from wild-type, D2-K317A, and D1-N87A *Synechocystis* cells were suspended in a buffer containing 8.4 mM sodium bicarbonate, 1 M sucrose, 10 mM $CaCl_2$, 200 mM NaCl, and 50 mM MES-NaOH (pH 6.50). DCBQ and K_3FeCN_6 were also added to final concentrations of 500 μM and 1 mM, respectively, before taking measurements. The resulting period-four oscillations were analyzed by fitting the data with the VZAD model using the BOBYQA nonlinear optimization algorithm [59].

2.4. EPR measurements

To study the effect of bicarbonate on the structure of the OEC, EPR samples of D2-K317A *Synechocystis* PSII core complexes were prepared in 8.4 mM sodium bicarbonate, 50 mM MES-NaOH (pH 6.5), 10% (*v*/v) glycerol, 1.2 M betaine, 20 mM $CaCl_2$, 5 mM $MgCl_2$, and 1 mM EDTA. The samples were then concentrated to 1 mg of Chl/mL using Amicon centrifugal cells having a 100 kDa cutoff. The S_2 state was generated by illuminating the sample with a Xe lamp in a 200 K acetone/dry ice bath. The measurements were performed by using a Bruker ELEXSYS E500 spectrometer using a SHQ resonator and an Oxford ESR-900 continuous flow cryostat at 7.5 K. The following EPR parameters were used for recording the spectra: microwave frequency, 9.38 GHz; modulation frequency, 100 kHz; modulation amplitude, 19.95 G; microwave power, 5 mW; sweep time, 84 s; conversion time, 41 ms; time constant, 82 ms. Each spectrum is the average of two scans.

3. Results and discussion

We have investigated the ability of bicarbonate to restore function lost by site-directed substitution of amino-acid residues in the second shell surrounding the OEC. The D2-K317A [41], D1-D61A [40] and CP43-R357K [55] mutations significantly impair oxygen-evolution activity and were investigated to assess whether the loss of function may be due to a role of these residues in the proton-transport process. The D1-N87A mutation alters chloride binding by perturbing the hydrogen-bonding network surrounding the OEC, but does not cause significant loss of oxygen-evolution activity [54]; D1-N87A PSII core complexes were used as a control to the understand the specificity of the bicarbonate effect.

The steady-state oxygen-evolution activity was measured with and without added bicarbonate for His-tagged *Synechocystis* (wild type) PSII core complexes and PSII core complexes isolated from four single-point PSII mutants: D2-K317A, D1-N87A, D1-D61A and CP43-R357K (Fig. 3 and Fig. S2). We investigated PSII core complexes isolated from these mutants rather than intact cells in order to exclude the indirect effect of bicarbonate in carbon concentrating mechanisms that is observed in cells [13,60]. To assess the activity of PSII with a low concentration of bicarbonate, the PSII core complexes were assayed in Assay Buffer containing 1 M sucrose, 60 mM Cl^-, 5 mM Ca^{2+} and MES-NaOH (pH 6.5). We also measured the dependence of the activity on the bicarbonate concentration over the range from 0 to 20 mM (Fig. S1). The effect of bicarbonate is maximal at concentrations of 8 mM and higher. Therefore, further studies of the effect of bicarbonate were done in Assay Buffer with the addition of 8.4 mM sodium bicarbonate.

We observe that bicarbonate selectively increases the oxygen-evolution activity of D2-K317A PSII core complexes by 22–25% at concentrations greater than 8.4 mM. However, added bicarbonate does not increase the oxygen-evolution activity of wild-type PSII core complexes or PSII core complexes with the D1-N87A, D1-D61A or CP43-R357K substitution (Fig. 3 and Fig. S2).

To investigate the S-state cycling efficiency of the PSII core complexes, we carried out flash-induced polarographic measurements (Fig. 4A). In D2-K317A PSII core complexes without added bicarbonate,

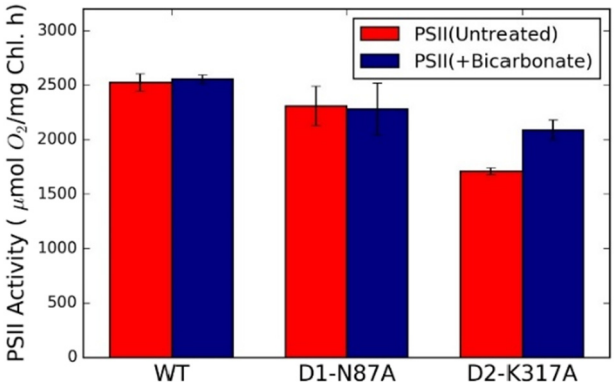

Fig. 3. Comparison of the PSII activity in wild-type (WT), D1-N87A and D2-K317A PSII core complexes. The assays were carried out in a buffer containing 1 M sucrose, 60 mM Cl^-, 5 mM Ca^{2+} and 50 mM MES-NaOH (pH 6.5) marked in red, or in the presence of an additional 8.4 mM sodium bicarbonate marked in dark blue. PPBQ and $K_3[FeCN]_6$ have been used as electron acceptors. The PSII activities are the average of 3–4 replicates with standard errors shown.

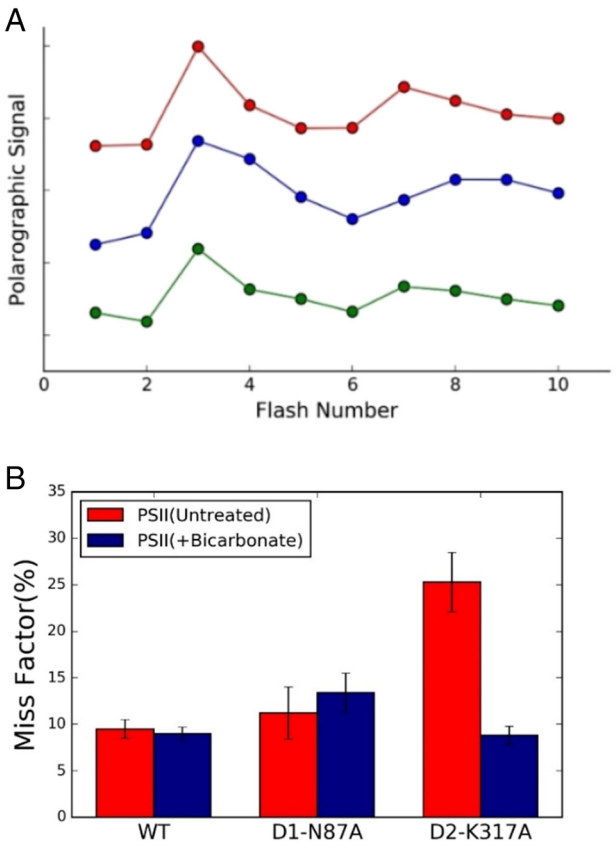

Fig. 4. A.Comparison of the flash-induced O_2 yields of wild-type PSII core complexes in the absence of added bicarbonate (red), D2-K317A PSII core complexes in the absence of added bicarbonate (blue) and D2-K317A PSII core complexes in the presence of 8.4 mM $NaHCO_3$ (green). The data points are offset vertically for clarity.
B. Comparison of the miss factors in wild-type (WT), D1-N87A and D2-K317A PSII core complexes (red) and in the presence of an additional 8.4 mM $NaHCO_3$ marked in dark blue. The miss factors are the average of 3–4 replicates with standard errors shown.

the period-four oscillation of oxygen evolution is perturbed relative to wild-type PSII core complexes. However, upon the addition of 8.4 mM bicarbonate, the period-four oscillation of oxygen evolution for D2-K317A PSII core complexes is similar to that observed for wild-type PSII

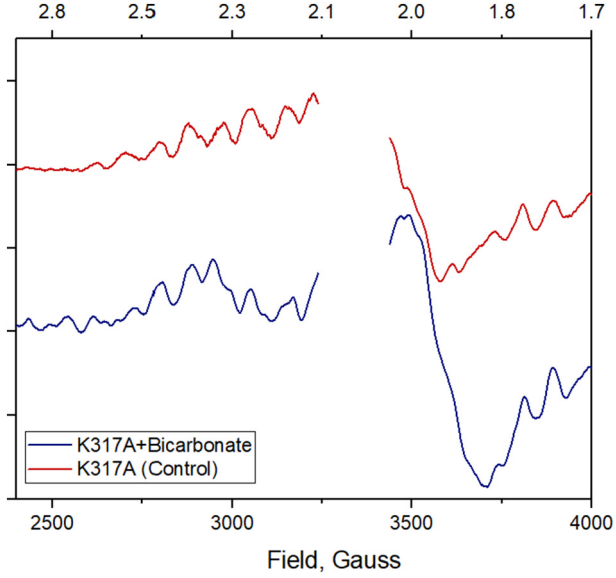

Fig. 5. S_2 state-minus-S_1 state difference EPR spectra of D2-K317A PSII core complexes: Untreated (red) and in the presence of 8.4 mM $NaHCO_3$ (blue). The g values are marked on the top of the graph.

core complexes. We analyzed the oscillations using the VZAD model to determine the miss factors [59]. Without added bicarbonate, the miss factor for D2-K317A PSII core complexes is 25% compared to 9% for His-tagged wild-type PSII core complexes, indicating impaired S-state cycling in the D2-K317A mutated PSII sample (Fig. 4B). The addition of 8.4 mM bicarbonate, reduces the miss factor of D2-K317A PSII to 9%, similar to that of wild-type PSII, whereas the addition of bicarbonate does not change the miss factor for either wild-type PSII core complexes or D1-N87A PSII core complexes (Fig. 4B); prior work has shown that the D1-N87A substitution does not perturb the period-four oscillation of oxygen evolution [54].

To understand the effect of bicarbonate treatment on the structure of the OEC, we studied the EPR spectra of the S_2 state of PSII core complexes isolated from the D2-K317A mutant strain. A 18–22 multi-line spectrum centered at g = 2, with an average hyperfine line spacing of 87.5 G is observed for D2-K317A PSII core complexes both in the presence and absence of added bicarbonate, indicating that bicarbonate does not alter the structure of the OEC (Fig. 5). Alterations to the structure of the OEC, such as those caused by the D1-S169A mutation, are revealed by significant differences in the average hyperfine line spacing (Fig. S3). These results support the conclusion that bicarbonate ions act as mobile proton carriers when added to PSII core complexes, in good agreement with the previous proposal by Messinger and co-workers [21].

We also studied the effect of bicarbonate on other site-directed mutated PSII core complexes with amino-acid substitutions proposed to affect the proton-transfer process. The D1-D61A mutation was selected because the D1-Asp61 residue has been proposed as a proton acceptor [40]. We observed that bicarbonate does not restore the oxygen-evolution activity of D1-D61A PSII core complexes (Fig. S2). We also studied the effect of bicarbonate on CP43-R357K PSII core complexes. The CP43-Arg357 residue has been previously proposed as a proton acceptor for proton-coupled electron transfer reactions of the OEC [2,61]. However, bicarbonate does not enhance the lowered oxygen-evolution activity observed in CP43-R357K PSII core complexes (Fig. S2).

Previous studies of D1-D61A PSII have shown that substantial oxygen-evolution activity is present in the absence of the D1-Asp61 residue [40], which was explained by the suggestion that there is an alternate proton-transfer pathway that can function in the absence of the native proton acceptor. Similarly, substantial activity remains when

CP43-Arg357 is substituted by Lys [55], which was explained by the suggestion that Lys can function as a base to accept protons in the absence of the native Arg proton acceptor. Our observation that bicarbonate does not restore the oxygen-evolution activity of D1-D61A or CP43-R357K PSII core complexes would be consistent with these proposals if bicarbonate does not act as an effective proton-transfer agent when compared to the alternate proton-transfer pathway(s). Further, an additional role of D1-Asp61 and CP43-Arg357 in substrate water transfer or maintaining the stability of PSII can't be ruled out. It is of interest that both D1-Asp61 and CP43-Arg357 have been proposed to function as proton acceptors in the proton-coupled electron transfer (PCET) reactions associated with advancement of the S states and the water-oxidation chemistry [2,40,55,61]. This raises the possibility that bicarbonate cannot restore the oxygen-evolution activity when D1-Asp61 or CP43-Arg357 are substituted by other residues because bicarbonate can only function as a mobile proton-transfer reagent and cannot restore the structural and chemical properties of the base required for a PCET reaction.

These studies indicate that bicarbonate acts as a mobile proton-transfer reagent and can be used to identify the single point mutations that function primarily to block the proton-transfer pathway. In D2-K317A PSII, the hydrogen-bonding interactions between chloride, D2-K317 and D1-D61 are perturbed, thereby inhibiting proton transfer [62]. The presence of mobile bicarbonate can, thereby, act as a suitable chemical-rescue agent to ensure efficient proton transfer to the lumen (Fig. 6). Similar effects are not observed for D2-K317A PSII core complexes in the presence of other anions like Br^- or NO_3^- (Fig. S4), thus supporting the conclusion that bicarbonate does not act as an alternate anion that can substitute for chloride but rather functions as a mobile proton acceptor.

4. Conclusions

This study provides direct experimental evidence that the proton relay to the lumen is blocked in D2-K317A PSII core complexes. Bicarbonate can act as an exogenous proton carrier, providing an alternate route of proton transfer, thereby resulting in improved oxygen-evolution activity. Our studies provide new insight on the application of bicarbonate as a chemical-rescue agent to recover the activity of single-point mutated PSII. Bicarbonate as a chemical-rescue agent thereby further supports a function of bicarbonate on the donor side of the PSII to aid in proton transfer. Here, we also demonstrate, as a proof-of-concept, how the bicarbonate chemical-rescue mechanism can be utilized to identify single-point mutations of PSII that affect the proton-transfer process to the lumen. The identification of the amino-acid residues that function in the proton-transfer process will subsequently help to reveal the role of the channels of waters and hydrogen-bonded amino-acid residues around the OEC, thereby providing us with a more comprehensive understanding of the water-oxidation mechanism.

Abbreviations

Chl	chlorophyll
D1	D1 polypeptide of PSII
D2	D2 polypeptide of PSII
DCBQ	2,6-dichloro-*p*-benzoquinone
EPR	electron paramagnetic resonance
OEC	oxygen-evolving complex
Pheo	Pheophytin
PPBQ	2-phenyl-1,4-benzoquinone
PSII	photosystem II
Y_Z	tyrosine Z

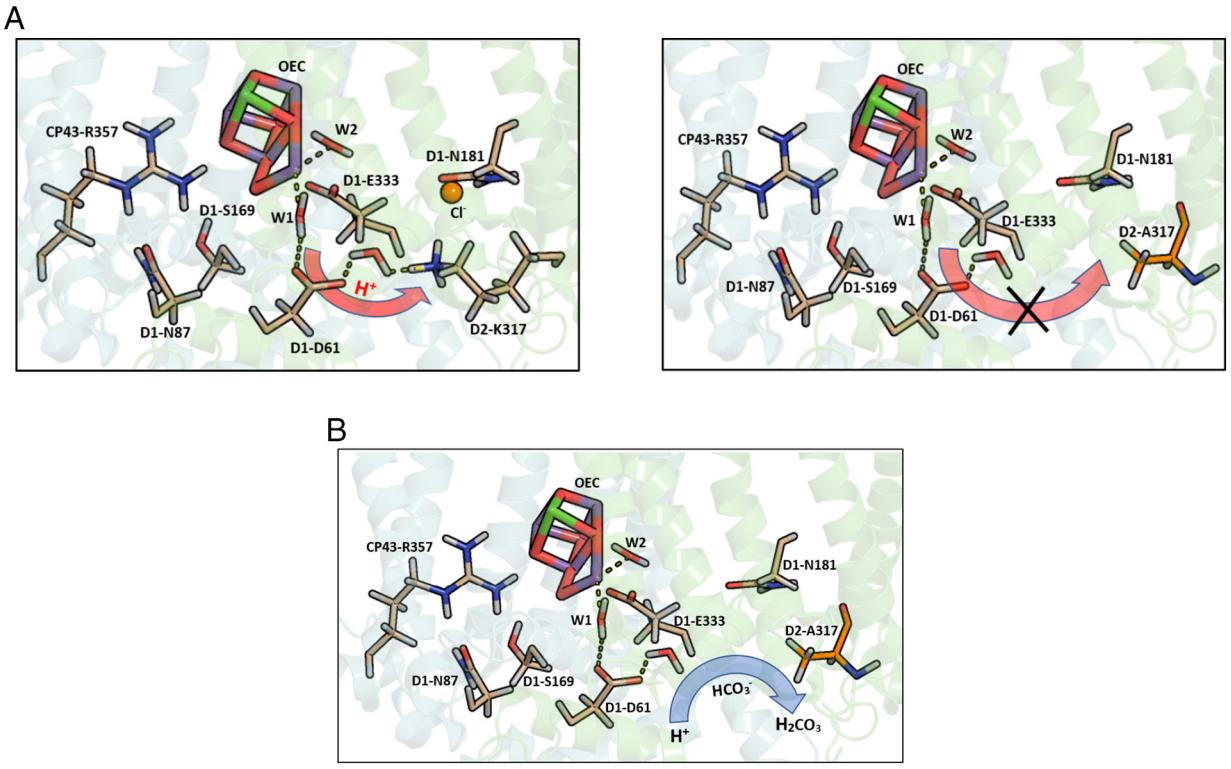

Fig. 6. A.(left side) Schematic depicting the proton-transfer process of wild-type PSII. (right side) Schematic depicting that the proton-transfer process is blocked in D2-K317A PSII. The C atoms are marked in pink, O atoms are marked in red, Mn in purple, N in blue, Ca in green and the Cl^- ion in orange. The alanine 317 residue in D2-K317A PSII is marked in orange. The dotted lines depict hydrogen-bonding interactions.
B. Schematic depicting the recovered proton transfer of D2-K317A PSII in the presence of bicarbonate. The O atoms are marked in red, Mn in purple, N in blue and Ca in green. The dotted lines depict hydrogen-bonding interactions. The alanine 317 residue is marked in orange.

Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Acknowledgements

This work was supported by grants from the Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences. Oxygen-release and EPR studies were supported by Grant DE-FG02-05ER15646 (to G.W.B.). Mutant construction was supported by Grant DE-SC0005291 (to R.J.D.).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbabio.2019.06.014.

References

[1] L. Vogt, D.J. Vinyard, S. Khan, G.W. Brudvig, Oxygen-evolving complex of photosystem II: an analysis of second-shell residues and hydrogen-bonding networks, Curr. Opin. Chem. Biol. 25 (2015) 152–158.
[2] J.P. McEvoy, G.W. Brudvig, Water-splitting chemistry of photosystem II, Chem. Rev. 106 (2006) 4455–4483.
[3] J. Kern, R. Chatterjee, I.D. Young, F.D. Fuller, L. Lassalle, M. Ibrahim, S. Gul, T. Fransson, A.S. Brewster, R. Alonso-Mori, Structures of the intermediates of Kok's photosynthetic water oxidation clock, Nature 563 (2018) 421–425.
[4] M. Perez-Navarro, F. Neese, W. Lubitz, D.A. Pantazis, N. Cox, Recent developments in biological water oxidation, Curr. Opin. Chem. Biol. 31 (2016) 113–119.
[5] D.J. Vinyard, G.W. Brudvig, Progress toward a molecular mechanism of water oxidation in photosystem II, Annu. Rev. Phys. Chem. 68 (2017) 101–116.
[6] D.A. Pantazis, Missing pieces in the puzzle of biological water oxidation, ACS Catal. 8 (2018) 9477–9507.
[7] K. Saito, A.W. Rutherford, H. Ishikita, Mechanism of tyrosine D oxidation in photosystem II, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 7690–7695.
[8] M. Suga, F. Akita, K. Hirata, G. Ueno, H. Murakami, Y. Nakajima, T. Shimizu, K. Yamashita, M. Yamamoto, H. Ago, Native structure of photosystem II at 1.95 Å resolution viewed by femtosecond X-ray pulses, Nature 517 (2015) 99–103.
[9] N. Cox, M. Retegan, F. Neese, D.A. Pantazis, A. Boussac, W. Lubitz, Electronic structure of the oxygen-evolving complex in photosystem II prior to OO bond formation, Science 345 (2014) 804–808.
[10] B. Kok, B. Forbush, M. McGloin, Cooperation of charges in photosynthetic O_2 evolution-I. a linear four step mechanism, Photochem. Photobiol. 11 (1970) 457–475.
[11] R. Pokhrel, G.W. Brudvig, Oxygen-evolving complex of photosystem II: correlating structure with spectroscopy, Phys. Chem. Chem. Phys. 16 (2014) 11812–11821.
[12] F. Müh, A. Zouni, The nonheme iron in photosystem II, Photosynth. Res. 116 (2013) 295–314.
[13] D. Shevela, J.J. Eaton-Rye, J.-R. Shen, Photosystem II and the unique role of bicarbonate: a historical perspective, Biochim. Biophys. Acta 1817 (2012) 1134–1151.
[14] J.J. Van Rensen, Role of bicarbonate at the acceptor side of photosystem II, Photosynth. Res. 73 (2002) 185–192.
[15] I. Ghosh, G. Banerjee, C.J. Kim, K. Reiss, V.S. Batista, R.J. Debus, G.W. Brudvig, D1-S169A substitution of photosystem II perturbs water oxidation, Biochemistry 58 (2019) 1379–1387.
[16] O. Warburg, Prefatory chapter, Annu. Rev. Biochem. 33 (1964) 1–15.
[17] A. Stemler, Bicarbonate ion as a critical factor in photosynthetic oxygen evolution, Plant Phys. 52 (1973) 119–123.
[18] T. Cardona, A. Sedoud, N. Cox, A.W. Rutherford, Charge separation in photosystem II: a comparative and evolutionary overview, Biochim. Biophys. Acta 1817 (2012) 26–43.
[19] F. Müh, C. Glöckner, J. Hellmich, A. Zouni, Light-induced quinone reduction in photosystem II, Biochim. Biophys. Acta 1817 (2012) 44–65.
[20] K. Tikhonov, D. Shevela, V. Klimov, J. Messinger, Quantification of bound bicarbonate in photosystem II, Photosynthetica (2017) 1–7.
[21] S. Koroidov, D. Shevela, T. Shutova, G. Samuelsson, J. Messinger, Mobile hydrogen carbonate acts as proton acceptor in photosynthetic water oxidation, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 6299–6304.
[22] D.J. Blubaugh, The Molecular Mechanism of the Bicarbonate Effect at the Plastoquinone Reductase Site of Photosynthesis, Molecular Biology of Photosynthesis, Springer, Dordrecht, 1988, pp. 441–484.
[23] R. Hienerwadel, C. Berthomieu, Bicarbonate binding to the non-heme iron of photosystem II, investigated by Fourier transform infrared difference spectroscopy and ^{13}C-labeled bicarbonate, Biochemistry 34 (1995) 16288–16297.
[24] N. Cox, L. Jin, A. Jaszewski, P.J. Smith, E. Krausz, A.W. Rutherford, R. Pace, The semiquinone-iron complex of photosystem II: structural insights from ESR and theoretical simulation; evidence that the native ligand to the non-heme iron is carbonate, Biophys. J. 97 (2009) 2024–2033.
[25] A. Sedoud, L. Kastner, N. Cox, S. El-Alaoui, D. Kirilovsky, A.W. Rutherford, Effects of formate binding on the quinone–iron electron acceptor complex of photosystem II, Biochim. Biophys. Acta 1807 (2011) 216–226.
[26] K. Saito, A.W. Rutherford, H. Ishikita, Mechanism of proton-coupled quinone reduction in photosystem II, Proc. Natl. Acad. Sci. U. S. A. 110 (2013) 954–959.
[27] K. Brinkert, S. De Causmaecker, A. Krieger-Liszkay, A. Fantuzzi, A.W. Rutherford, Bicarbonate-induced redox tuning in photosystem II for regulation and protection, Proc. Natl. Acad. Sci. U. S. A. 113 (2016) 12144–12149.
[28] G. Govindjee, Bicarbonate-reversible inhibition of plastoquinone reductase in photosystem II, Z. Naturforsch. C 48 (1993) 251–258.
[29] I.L. McConnell, J.J. Eaton-Rye, J.J. van Rensen, Regulation of Photosystem II Electron Transport by Bicarbonate, Photosynthesis, Springer, Dordrecht, 2012, pp. 475–500.
[30] V. Klimov, R. Hulsebosch, S. Allakhverdiev, H. Wincencjusz, H. Van Gorkom, A. Hoff, Bicarbonate may be required for ligation of manganese in the oxygen-evolving complex of photosystem II, Biochemistry 36 (1997) 16277–16281.
[31] D.N. Shevela, A.A. Khorobrykh, V.V. Klimov, Effect of bicarbonate on the water-oxidizing complex of photosystem II in the super-reduced S-states, Biochim. Biophys. Acta 1757 (2006) 253–261.
[32] G. Dismukes, V. Klimov, S. Baranov, Y.N. Kozlov, J. DasGupta, A. Tyryshkin, The origin of atmospheric oxygen on earth: the innovation of oxygenic photosynthesis, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 2170–2175.
[33] S.V. Baranov, A.M. Tyryshkin, D. Katz, G.C. Dismukes, G.M. Ananyev, V.V. Klimov, Bicarbonate is a native cofactor for assembly of the manganese cluster of the photosynthetic water oxidizing complex. Kinetics of reconstitution of O_2 evolution by photoactivation, Biochemistry 43 (2004) 2070–2079.
[34] S.V. Baranov, G.M. Ananyev, V.V. Klimov, G.C. Dismukes, Bicarbonate accelerates assembly of the inorganic core of the water-oxidizing complex in manganese-depleted photosystem II: a proposed biogeochemical role for atmospheric carbon dioxide in oxygenic photosynthesis, Biochemistry 39 (2000) 6060–6065.
[35] C. Aoyama, H. Suzuki, M. Sugiura, T. Noguchi, Flash-induced FTIR difference spectroscopy shows no evidence for the structural coupling of bicarbonate to the oxygen-evolving Mn cluster in photosystem II, Biochemistry 47 (2008) 2760–2765.
[36] Y. Umena, K. Kawakami, J.-R. Shen, N. Kamiya, Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å, Nature 473 (2011) 55–60.
[37] D. Shevela, J.-H. Su, V. Klimov, J. Messinger, Hydrogencarbonate is not a tightly bound constituent of the water-oxidizing complex in photosystem II, Biochim. Biophys. Acta 1777 (2008) 532–539.
[38] G. Ulas, G. Olack, G.W. Brudvig, Evidence against bicarbonate bound in the O_2-evolving complex of photosystem II, Biochemistry 47 (2008) 3073–3075.
[39] N. Cox, J. Messinger, Reflections on substrate water and dioxygen formation, Biochim. Biophys. Acta 1827 (2013) 1020–1030.
[40] J. Clausen, R.J. Debus, W. Junge, Time-resolved oxygen production by PSII: chasing chemical intermediates, Biochim. Biophys. Acta 1655 (2004) 184–194.
[41] R. Pokhrel, R.J. Service, R.J. Debus, G.W. Brudvig, Mutation of lysine 317 in the D2 subunit of photosystem II alters chloride binding and proton transport, Biochemistry 52 (2013) 4758–4773.
[42] R.J. Debus, FTIR studies of metal ligands, networks of hydrogen bonds, and water molecules near the active site Mn_4CaO_5 cluster in photosystem II, Biochim. Biophys. Acta 1847 (2015) 19–34.
[43] F.M. Ho, Structural and mechanistic investigations of photosystem II through computational methods, Biochim. Biophys. Acta 1817 (2012) 106–120.
[44] S. Ferguson-Miller, C. Hiser, J. Liu, Gating and regulation of the cytochrome c oxidase proton pump, Biochim. Biophys. Acta 1817 (2012) 489–494.
[45] J. Geng, A. Liu, Heme-dependent dioxygenases in tryptophan oxidation, Arch. Biochem. Biophys. 544 (2014) 18–26.
[46] C.M. Maupin, N. Castillo, S. Taraphder, C. Tu, R. McKenna, D.N. Silverman, G.A. Voth, Chemical rescue of enzymes: proton transfer in mutants of human carbonic anhydrase II, J. Am. Chem. Soc. 133 (2011) 6223–6234.
[47] H. Cheap, J. Tandori, V. Derrien, M. Benoit, P. de Oliveira, J. Koepke, J. Lavergne, P. Maroti, P. Sebban, Evidence for delocalized anticooperative flash induced proton binding as revealed by mutants at the M266His iron ligand in bacterial reaction centers, Biochemistry 46 (2007) 4510–4521.
[48] P. Sebban, P. Maroti, M. Schiffer, D.K. Hanson, Electrostatic dominoes: long distance propagation of mutational effects in photosynthetic reaction centers of *Rhodobacter capsulatus*, Biochemistry 34 (1995) 8390–8397.
[49] P. Maróti, D.K. Hanson, M. Schiffer, P. Sebban, Long-range electrostatic interaction in the bacterial photosynthetic reaction centre, Nat. Struct. Biol. 2 (1995) 1057–1059.
[50] P. Maróti, Chemical rescue of H^+ delivery in proton transfer mutants of reaction center of photosynthetic bacteria, Biochim. Biophys. Acta 1860 (2019) 317–324.
[51] M. Paddock, P. Ädelroth, G. Feher, M. Okamura, J. Beatty, Determination of proton transfer rates by chemical rescue: application to bacterial reaction centers, Biochemistry 41 (2002) 14716–14725.
[52] E. Takahashi, C.A. Wraight, Small weak acids reactivate proton transfer in reaction centers from *Rhodobacter sphaeroides* mutated at AspL210 and AspM17, J. Biol. Chem. 281 (2006) 4413–4422.
[53] G. Ulas, G.W. Brudvig, Zwitterion modulation of O_2-evolving activity of cyanobacterial photosystem II, Biochemistry 49 (2010) 8220–8227.
[54] G. Banerjee, I. Ghosh, C.J. Kim, R.J. Debus, G.W. Brudvig, Substitution of the D1-Asn87 site in photosystem II of cyanobacteria mimics the chloride-binding characteristics of spinach photosystem II, J. Biol. Chem. 293 (2018) 2487–2497.
[55] H.J. Hwang, P. Dilbeck, R.J. Debus, R.L. Burnap, Mutation of arginine 357 of the CP43 protein of photosystem II severely impairs the catalytic S-state cycle of the H_2O oxidation complex, Biochemistry 46 (2007) 11987–11997.
[56] H.-A. Chu, R.J. Debus, G.T. Babcock, D1-Asp170 is structurally coupled to the

oxygen evolving complex in photosystem II as revealed by light-induced Fourier transform infrared difference spectroscopy, Biochemistry 40 (2001) 2312–2316.
[57] M.A. Strickler, L.M. Walker, W. Hillier, R.J. Debus, Evidence from biosynthetically incorporated strontium and FTIR difference spectroscopy that the C-terminus of the D1 polypeptide of photosystem II does not ligate calcium, Biochemistry 44 (2005) 8571–8577.
[58] D.J. Vinyard, G.W. Brudvig, Insights into substrate binding to the oxygen-evolving complex of photosystem II from ammonia inhibition studies, Biochemistry 54 (2015) 622–628.
[59] D.J. Vinyard, C.E. Zachary, G. Ananyev, G.C. Dismukes, Thermodynamically accurate modeling of the catalytic cycle of photosynthetic oxygen evolution: a mathematical solution to asymmetric Markov chains, Biochim. Biophys. Acta 1827 (2013) 861–868.
[60] D.T. Hanson, L.A. Franklin, G. Samuelsson, M.R. Badger, The *Chlamydomonas reinhardtii* cia3 mutant lacking a thylakoid lumen-localized carbonic anhydrase is limited by CO_2 supply to rubisco and not photosystem II function *in vivo*, Plant Physiol. 132 (2003) 2267–2275.
[61] E.M. Sproviero, J.A. Gascon, J.P. McEvoy, G.W. Brudvig, V.S. Batista, Quantum mechanics/molecular mechanics structural models of the oxygen-evolving complex of photosystem II, Curr. Opin. Struct. Biol. 17 (2007) 173–180.
[62] R. Pokhrel, I.L. McConnell, G.W. Brudvig, Chloride regulation of enzyme turnover: application to the role of chloride in photosystem II, Biochemistry 50 (2011) 2725–2734.