Post-translational amino acid conversion in photosystem II as a possible origin of photosynthetic oxygen evolution

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Photosynthetic oxygen evolution is performed at the Mn cluster in photosystem II (PSII). The advent of this reaction on ancient Earth changed its environment by generating an oxygenic atmosphere. However, how oxygen evolution originated during the PSII evolution remains unknown. Here, we characterize the site-directed mutants at the carboxylate ligands to the Mn cluster in cyanobacterial PSII. A His residue replaced for D1-D170 is found to be post-translationally converted to the original Asp to recover oxygen evolution. Gln/Asn residues in the mutants at D1-E189/D1-D342 are also converted to Glu/Asp, suggesting that amino-acid conversion is a common phenomenon at the ligand sites of the Mn cluster. We hypothesize that post-translational generation of carboxylate ligands in ancestral PSII could have led to the formation of a primitive form of the Mn cluster capable of partial water oxidation, which could have played a crucial role in the evolutionary process of photosynthetic oxygen evolution.
In oxygenic photosynthesis, oxygen evolution by water oxidation is carried out in the oxygen-evolving complex (OEC)\textsuperscript{1,2}, which has an inorganic catalytic core of Mn\textsubscript{4}CaO\textsubscript{6} (Mn cluster), formed in photosystem II (PSII) through a photoassembly process called photoactivation\textsuperscript{3-5} (Fig. 1). The water oxidation not only provides electrons necessary for CO\textsubscript{2} fixation, but also plays a significant role in sustenance of the environment and life on Earth as a source of oxygen in the atmosphere. In the OEC, two water molecules are oxidized to one oxygen molecule and four protons through a cycle of five intermediates (S\textsubscript{0}-S\textsubscript{4} states)\textsuperscript{6-8}, which are advanced by light-induced electron transfer. All extant oxyphophotrophs have essentially the identical OEC structure with conserved amino acid residues including ligands to the Mn and Ca ions, i.e., six carboxylate and one histidine ligands from D170, E189, H332, E333, D342, and A344 (C-terminus) of the D1 protein and E354 of the CP43 protein (Fig. 1a)\textsuperscript{6}.

The advent of photosynthetic oxygen evolution on ancient Earth was a key event in the co-evolution of Earth and life. It produced an oxidative atmosphere and promoted the evolution of aerobic life followed by its extensive diversification\textsuperscript{7,8}. It is unclear when photosynthetic oxygen evolution originated on Earth; the estimates by geochemical and phylogenetic studies span widely from 3.8 to 2.3 billion years ago\textsuperscript{9-16}. Recent evolutionary studies of the PSII proteins by Cardona and coworkers suggested that a functional OEC, nearly identical to that found in extant PSII, developed and the water oxidation reaction was optimized in the early stage during the evolution of life in the early Archean. However, how the ligand environment of the Mn cluster was formed in photosystem II (PSII) through a photoassembly process called photoactivation\textsuperscript{3} (Fig. 1). The water oxidation intermediate in the S\textsubscript{1} state comprises Mn\textsuperscript{4+}CaO\textsubscript{5} and 2H\textsubscript{2}O as its immediate precursors, which was obtained by QM/MM calculation based on the X-ray crystallographic structure\textsuperscript{6}.

Here, we investigate the mechanism of the amino-acid conversion in mutants at the ligands to the Mn cluster using LC-MS and FTIR analyses. We analyze the PSII from D1-D170H cells incorporated with isotope-labeled histidine to clarify whether the conversion occurs at the protein level after translation. Furthermore, we examine the conversion in mutants at other carboxylate ligands, D1-E189 and D1-D342. The obtained results provide insights into the evolutionary process of photosynthetic water oxidation and the origin of oxygen in the ancient atmosphere.

**Results**

**Post-translational conversion of His to Asp.** The mechanism of the His—Asp conversion in the D1-D170H mutant was first examined at the RNA level. The sequence of psbA2 mRNA obtained from mixotrophically grown D1-D170H cells was determined from its cDNA. The codon for the amino acid at position 170 was CAT for His, and no trace of a codon for Asp (GAT/GAC) was detected at this position (Supplementary Fig. 1). This indicates that no modification occurred in the mRNA of the psbA2 gene after transcription in D1-D170H cells.

Modification at the protein level was next examined using isotope-labeled histidine. D1-D170H cells were incorporated with \textsuperscript{13}C-labeled histidine and isotope labeling of the Asp residue after the conversion was examined. Flash-induced FTIR difference spectra upon the S\textsubscript{1} → S\textsubscript{2} transition (S\textsubscript{2}/S\textsubscript{1} difference) measured with the PSII complexes from D1-D170H cells incorporated with unlabeled (\textsuperscript{12}C-His)D170H and \textsuperscript{13}C-labeled (\textsuperscript{13}C-His)D170H) histidine were compared (Fig. 2a). Clear \textsuperscript{13}C-induced changes were observed in the prominent bands in 1600–1500 and 1450–1300 cm\textsuperscript{−}1, typical regions of the asymmetric and symmetric COO\textsuperscript{−} stretching vibrations, respectively, of the carboxylate groups around the Mn cluster\textsuperscript{21}. The \textsuperscript{12}C-minus-\textsuperscript{13}C double difference spectrum more clearly revealed the changes showing bands at 1586/1566/1551/1529 cm\textsuperscript{−}1 and 1399/1376/1362/1320 cm\textsuperscript{−}1, suggesting the \textsuperscript{13}C-induced downshifts of differential signals in the S\textsubscript{2}/S\textsubscript{1} difference spectrum. The spectral feature in the symmetric COO\textsuperscript{−} region was well reproduced by the quantum mechanics/molecular mechanics (QM/MM) calculations (Fig. 2b), in which the carbon atoms of D1-D170 were selectively labeled with \textsuperscript{13}C. The calculated normal modes (Fig. 2b, sticks) revealed that the experimental bands at 1399(S\textsubscript{i})/1362(S\textsubscript{j}) cm\textsuperscript{−}1 and 1376(S\textsubscript{x})/1320(S\textsubscript{y}) cm\textsuperscript{−}1 are attributed to the vibrations of unlabeled and \textsuperscript{13}C-labeled D170, respectively.

The \textsuperscript{13}C labeling of D1-D170 converted from His was further examined by LC-MS analysis (Fig. 2c, d). In both \textsuperscript{12}C-His\textsuperscript{7} D170H and \textsuperscript{13}C-His\textsuperscript{7}D170H cells, the H170 residue in 58-59% and 3-4% of the D1 proteins was converted to Asp and Asn, respectively (Fig. 2d). In the D1 protein from \textsuperscript{13}C-His\textsuperscript{7}D170H cells, the MS spectral peaks of \textsuperscript{13}C\textsubscript{6}-labeled D170 (Fig. 2c) and N170 (Supplementary Fig. 2b) in addition to the peak of \textsuperscript{13}C\textsubscript{6}-labeled H170 (Supplementary Fig. 2a) were detected. The MS chromatogram bands (Fig. 2c and Supplementary Fig. 2) showed that 66% of the His, Asp, and Asn residues at D1-170, in total, was labeled with \textsuperscript{13}C (Fig. 2d), which is similar to ~61% \textsuperscript{13}C labeling at D1-H332 as a histidine ligand to the Mn cluster and at D1-H190 interacting with Y\textsubscript{Z}, indicating that 60–70% of His residues were labeled with \textsuperscript{13}C in \textsuperscript{13}C-His\textsuperscript{7}D170H cells. It is
noted that no appreciable conversion of the D1-H332 ligand to Asp was detected. Thus, from the FTIR, QM/MM, and LS-MS results together with the RNA analysis, it is definitely concluded that D1-H170 in the D1-D170H mutant was post-translationally converted to the original Asp residue (and to Asn to a minor extent) at the protein level.

Involvement of light-induced oxidation of Mn. We previously showed that phototrophic growth is necessary for this His→Asp conversion. Although under heterotrophic condition with herbicide DCMU and dim light, the His→Asp conversion hardly occurred (~3%; Fig. 2e, iii), the same dim-light condition in the absence of DCMU converted ~58% of H170 to Asp (Fig. 2e, ii and Supplementary Fig. 3), indicating the necessity of electron transfer in PSII for the conversion.

The involvement of a Mn ion(s) in the amino-acid conversion was (were) further examined. It was previously reported that *Synechocystis* sp. PCC 6803 cells can grow in Mn²⁺-depleted medium keeping the PSII level. We grew D1-D170H mutant cells under mixotrophic condition in Mn²⁺-depleted medium. The PSII complexes prepared from the thus obtained cells showed no O₂ evolution and LC-MS analysis detected only His at D1-170 without any trace of Asp (Fig. 2e, iv and Supplementary Fig. 4). These observations thus indicate that a Mn²⁺ ion(s) photo-oxidized in the OEC site is (are) directly involved in the mechanism of the His→Asp conversion.

Conversion at other ligand sites and amino acid dependence. We further examined the amino acid conversion at other ligand sites of the Mn cluster and dependence on the amino acid species. D1-E189 and D1-D342 were replaced with corresponding amide.
residues (E189Q and D342N) and a smaller Ala residue (E189A and D342A). The PSII complexes from E189Q and D342N cells grown mixotrophically showed O2 evolution activities of 58 ± 3 and 44 ± 5% of the WT* PSII, whereas those from E189A and D342A cells showed no O2 activity (Fig. 3b). The S2/S1 FTIR difference spectra of the E189Q and D342N PSII (Fig. 3a) showed features very similar to those of the WT* PSII, which is consistent with the previous results23,24, but with amplitudes of 57 and 35%, respectively, on the protein basis (Fig. 3b). In contrast, the FTIR evolution activity (red bars) and the FTIR amplitude (ΔA between 1433 and 1397 cm⁻¹, blue bars) relative to those of WT* and the relative contents of amino acid residues at the mutation sites estimated by LC-MS analysis (green bars) in the PSII complexes from D1-E189Q, E189A, D342N, and D342A cells. The O2 evolution activities were presented as mean values ± standard deviation (n = 3), and the error of the FTIR amplitude was estimated from the root-mean-square noise level in the 1400-1390 cm⁻¹ region of the dark-minus-dark spectrum. Source data are provided as a Source Data file.

Fig. 3 FTIR and LC-MS analyses of the D1-E189Q/A and D1-D342N/A mutants. a S2/S1 FTIR difference spectra of the PSII complexes from (i) D1-E189Q (red line) and E189A (blue line) and (ii) D1-D342N (red line) and D342A (blue line) cells in comparison with the spectrum of WT* PSII (black lines). The amplitudes of the spectra were normalized based on the protein amounts estimated from the amide II band. The amplitudes of the spectra were normalized based on the D342A (blue line) cells in comparison with the spectrum of WT* PSII E189Q (red line) and E189A (blue line) and (ii) D1-D342N (red line) and D342A cells. The O2 evolution activities were presented as mean values ± standard deviation (n = 3), and the error of the FTIR amplitude was estimated from the root-mean-square noise level in the 1400-1390 cm⁻¹ region of the dark-minus-dark spectrum. Source data are provided as a Source Data file.

b

Discussion
In this study, we found that some amino acid residues at the ligand sites of the Mn cluster can be post-translationally converted to the original carboxylate residues. An oxidized Mn ion(s) produced by light-induced electron transfer is (are) most likely involved in the mechanism of this amino-acid conversion. In the photoactivation process, the first Mn²⁺ ion, probably bound to D1-D170 and D1-E1895,23, is oxidized to Mn³⁺, and then after the conformational change of the D1 C-terminal region, involving D1-E333 and D1-D3425, another Mn²⁺ is oxidized to Mn³⁺ to form a relatively stable bimetallic complex3,4. Further illumination oxidizes Mn ions to Mn³⁺/⁴⁺ to finally construct the Mn cluster with a Mn₄CaO₅ form (Fig. 1b).

Some cases of post-translational amino-acid conversion have been reported in vivo and in vitro26-29. One typical case is metal-catalyzed oxidation of a His residue by a hydroxyl radical induced by H₂O₂ with an aid of a metal ion, typically Fe²⁺ or Cu⁺, through Fenton-like reactions26,27. His is first converted to 2-oxohistidine and then to Asp or Asn. Mn²⁺ is also effective in producing a hydroxyl radical from H₂O₂, and some Mn oxide complexes supplemented with H₂O₂ have been reported to oxidize various organic compounds31,32. Indeed, H₂O₂ has been detected as a byproduct in water oxidation reactions of some modified OEC33. It is thus speculated that reactive oxygen species...
(ROS) such as H$_2$O$_2$ and a hydroxyl radical produced from water during photoactivation oxidizes a His residue at the ligand site to Asp or Asn (Fig. 4). Another case is deamidation of Asn and Glu residues to change into Asp and Glu, respectively$^{28,29}$. Deamidation is carried out by hydrolysis of amide groups to carboxylate groups, and in proteins the rate of deamidation strongly depends on the primary sequence and the 3D structure around the reacting residue$^{28}$. A relationship was also suggested between metal binding and deamidation in amyloidogenic proteins$^{29}$. It is thus possible that Mn binding at amide residues promotes deamidation to generate Glu/Asp ligands (Fig. 4).

The amino acid residues that are converted to carboxylate residues may not be limited to His and amide residues. It has been reported that mutations of D1-D170 to Tyr, Trp, Asn, His, Met, Glu, Arg, Val, Leu, and Ile, all of which have long side chains at least with a γ-carbon, retained partial O$_2$ evolution activity$^{34-36}$, suggesting the presence of the intact Mn cluster in some PSII complexes in the mutants (note that Glu replaced for D1-D170 may form a partially functional Mn cluster$^{37}$). In contrast, mutations of D1-D170 to shorter amino acid residues such as Ala and Ser did not show O$_2$ evolution$^{34,35}$. In addition, mutations of D1-E189 to Gln, Arg, Lys, Leu, and Ile, which have an aliphatic δ-carbon, largely restored the O$_2$ evolution, whereas mutations to residues having shorter aliphatic chains showed no or only a trivial amount of O$_2$ evolution$^{36,38}$. Furthermore, mutations of D1-D342 to Asn, His, and Glu having a γ-carbon showed some O$_2$ evolution$^{35,39}$, whereas the Ala mutant did not support O$_2$ evolution$^{39}$ in agreement with our result (Fig. 3). These observations can be explained by post-translational conversions from residues with side chains long enough to form Asp/Glu and the absence of conversion from those with shorter side chains. In particular, the presence of O$_2$ activity upon mutations of D170 or E189 to aliphatic residues such as Val, Lue, and Ile, which cannot ligate the Mn cluster, is understandable if these side chains are converted to the Asp/Glu side chains. These amino-acid conversions except for deamidation of Asn/Gln may also be promoted by ROS formed from water through Mn$^{3+}/4^+$ ions, although the exact conversion mechanism for each amino acid residue requires further investigations.

In the D170H, E189Q, and D342N mutants, relatively large amounts (>50%) of the D1 proteins with converted original amino acid ligands were accumulated in mixotrophically grown cells (Figs. 2 and 3). In the steady state of the turnover of the D1 protein in the cell culture of the mutants, where the total amount of the D1 protein is constant and the relative amounts of the converted functional and initial unconverted D1 proteins ([cD1] and [id1], respectively) are unchanged, their ratio ([cD1]/[id1]) is determined by the rate constant of conversion (k$_c$) relative to that of degradation of the converted D1 protein (k$_d$) ([cD1]/[id1] = k$_c$/k$_d$; Supplementary Fig. 7). The turnover of the D1 protein in the Synechocystis sp. PCC 6803 mutant with psbA2 as a single gene for the D1 protein (corresponding to our WT*) was previously shown to occur in several hours under the light of 100 μmol photons m$^{-2}$s$^{-1}$. Thus, the presence of comparable amounts of the converted and unconverted D1 proteins indicates that amino-acid conversion in the D170H, E189Q, and D342N mutants through ROS-mediated oxidation or deamidation has time constants of hours under the light of ~50 μmol photons m$^{-2}$s$^{-1}$ used in our experiments. In the oxidation by ROS, a variety of oxidized products, such as 2-oxo-histidine in the His→Asp conversion, would be formed as impaired D1 proteins. However, because non-functional D1 proteins degrade much faster than the functional D1 protein (i.e., large k$_d$)$^{40,41}$, they are hardly accumulated in cells. For the same reason, the D1 protein modified by the His→Asp conversion at D1-H332, an original His ligand to the Mn cluster, will not be accumulated in cells, because of the formation of a non-functional D1 protein$^{39}$.

![Heliobacterium modesticaldum](https://example.com/heliobacterium_modesticaldum.png)

**Fig. 5 Hypothetical mechanism of generation of a primitive OEC in ancestral PSII.** a. The ancestral PSII had a D/E residue(s) for binding of an initial Mn$^{2+}$ ion, which can be oxidized by light-induced electron transfer. Carboxylate ligands were generated by post-translational amino-acid conversion and some sort of Mn-oxide complex was formed in the OEC site to perform partial water oxidation. During this inefficient O$_2$ evolution, the ligand environment and proton and water pathways were optimized, and protection mechanisms against ROS were developed in PSII. “L” indicates non-carboxylic amino acid residues at the ligand positions. b. Completed OEC capable of efficient water oxidation.

Post-translational amino-acid conversions so far reported inactivate proteins in relevance to aging and diseases$^{27-29}$. The amino-acid conversion found in this study, which generates carboxylate ligands in OEC, is a very unique case that restores an enzymatic function, photosynthetic oxygen evolution, essential for life and its evolution.

One of the biggest questions in the evolution of photosynthesis is how the OEC originated in the ancestral PSII$^{15,17-19}$. Our data suggest that water oxidation could have originated before a fully complete ligand sphere had time to evolve in the early photosystem. We speculate that in the ancestral PSII, which has at least a single Mn binding site by an Asp/Glu residue(s), a Mn$_{3+}/4^+$ ion formed by light-induced electron transfer in the reaction center promoted post-translational conversion of nearby amino-acid residues to generate Asp/Glu ligands (Fig. 5). Such an initial metal binding site on the electron-donor side could have existed even in reaction centers at a very early stage of photosynthesis evolution. Indeed, evidence for such an ancient site may be found in extant homodimeric type I reaction centers. In the *Heliobacterium modesticaldum* type I reaction center, an exposed Ca-binding site was recently discovered with structural similarities to the OEC in
PSII, including two carboxylate ligands to the Ca atom, an Asp and the C-terminal carboxylic group. The presence of several carboxylate ligands with negative charges is significant for the formation of a functional Mn cluster not only by fixing the Mn/ Ca ions but also by tuning its redox potential. Recently, Chernen et al. showed light-induced formation of birnessite-type Mn(III,IV)-oxide nanoparticles in apo-PSII, and proposed that such a Mn-oxide particle was down-sized to develop the Mn cluster. Thus different types of high-valent Mn-oxide complexes in various sizes could be formed in the OEC site by light-induced electron transfer during the evolution of PSII. It has also been reported that many high-valent Mn-oxide complexes function as catalysts of water oxidation. It is thus presumed that some sort of photo-produced Mn-oxide complex was stabilized and functionalized by post-translationally generated carboxylate ligands in the ancestral PSII, and carried out partial oxygen evolution.

This inefficient water oxidation, however, would have been crucial in the further development of PSII. In addition to heterodimerization and optimization of the ligand environment, the proton-exit and water-divide pathways could have been formed around the Mn cluster to optimize the water oxidation reaction, while various protection mechanisms and repair systems would have been developed. It would also have stimulated the evolution of aerobic respiration and oxygen-related enzymes. Thus, the post-translational amino-acid conversion, beyond the central dogma, to generate the carboxylate ligands to the Mn cluster could have played a significant role in the evolution of photosynthetic water oxidation and that of aerobic life.

**Methods**

**Construction of site-directed mutants.** Site-directed mutagenesis of the psbA2 gene encoding the D1 subunit was performed in *Synechocystis* sp. PCC 6803. Plasmid pRN123, which involved the coding region of psbA2, was used as a parent vector for site-directed mutagenesis. The host *Synechocystis* strain, which lacks all of the three psbA genes (ΔpsbA1/ΔpsbA2/ΔpsbA3) and contains a hexahistidine tag attached to the C-terminus of the CP47 protein, was transformed with pRN123 to provide a control strain with the wild-type D1 protein (WT†). Mutation of D1-D170 to His (ΔD1-D170-His) was then introduced into ΔD1-D170 strain to provide a strain with a high-valent Mn-oxide complex (ΔD1-D170-His/ΔD1-D170-His). This strain was used for preparation of ΔD1-D170-His/ΔD1-D170-His. For a low-light condition, dim light was used (1 µmol photons m−2 s−1). The measurement was repeated 100 times for ΔD1-D170-His/ΔD1-D170-His and WT†. The sample temperature was kept at 10 °C by circulating cold water through a copper holder. FTIR difference spectra were measured using a Bruker Vertex 80 spectrophotometer (with the OPUS 7.8 software) with an MCT detector at 4 cm−1 resolution. A Ge filter (Andover, 430ILP-25) was placed in the infrared path to cut light at >2200 cm−1. FTIR spectra with 20 scans were recorded twice before and after single-flash illumination from a Nd:YAG laser (Quanta-Ray NDY-10/40: 532 nm, ~7 ns fwhm, ~150 mJ/flash). The measurements were repeated 16 times for 1.5 cm−1. The measurement was repeated 100 times for ΔD1-D170-His/ΔD1-D170-His and WT† with a dark interval of 2 min, and spectra were averaged. A difference spectrum of after-minus-before illumination provided the changes upon the S1 → S0 transition, while that of the two spectra after illumination represented the noise level.

**Mass spectrometry analysis.** Amino acid sequences of the D1 proteins were analyzed using mass spectrometry. PSII core complexes solubilized with 3% lithium lauryl sulfate in the presence of 75 mM dithiothreitol were subjected to SDS—PAGE. The isolated D1 protein was applied to nano-reversed-phase chromatography (nLC-MS/MS) after digestion with trypsin. The obtained cDNA, a DNA fragment (1.0 kbp) in the internal region of the *psbA2* gene, was amplified by PCR using a primer set of 5′-ACGGATCCTACGTGACTGAGGCAAGTCAAGGACGAGTGG-3′ and 5′-CAGCATTTTCTGCTACATGGGAATCCGAGTTCAGTGCC-3′. The amplified fragment was sequenced to confirm the proper genotypes of individual strains. The cDNA from D1-D170 cells was amplified by PCR using a primer set of 5′-ACGGATCCTACGTGACTGAGGCAAGTCAAGGACGAGTGG-3′ and 5′-CAGCATTTTCTGCTACATGGGAATCCGAGTTCAGTGCC-3′ using an ABI 3100 DNA sequencer.

**Quantum mechanics/molecular mechanics calculations.** The infrared spectra of the carboxylate groups in the OEC were simulated using quantum mechanics/molecular mechanics (QM/MM) calculations. The coordinates of heavy atoms in the Mn cluster, surrounding amino acid residues, water molecules, and two Cl− ions were used for the calculation.

**Figure captions**

1. Asp and the C-terminal carboxylic group.
2. The presence of several carboxylate ligands with negative charges is significant for the formation of a functional Mn cluster not only by fixing the Mn/ Ca ions but also by tuning its redox potential. Recently, Chernen et al. showed light-induced formation of birnessite-type Mn(III,IV)-oxide nanoparticles in apo-PSII, and proposed that such a Mn-oxide particle was down-sized to develop the Mn cluster. Thus different types of high-valent Mn-oxide complexes in various sizes could be formed in the OEC site by light-induced electron transfer during the evolution of PSII. It has also been reported that many high-valent Mn-oxide complexes function as catalysts of water oxidation. It is thus presumed that some sort of photo-produced Mn-oxide complex was stabilized and functionalized by post-translationally generated carboxylate ligands in the ancestral PSII, and carried out partial oxygen evolution.

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ions within 20 Å from the Mn cluster were extracted from the X-ray structure (PDB ID: 4UB6) of PSII in the S1 state. Hydrogen atoms were optimized using the AMBER force field55 fixing all heavy atoms. QM/MM calculations were carried out by the ONIOM method56 with the electronic embedding scheme in the Gaussian 16 program package57. The QM region (Supplementary Fig. 8) consists of the Mn cluster, Cl-1, seven amino acid ligands (D1-D170, D1-E189, D1-H332, D1-E333, D1-D334, D1-A344, CP43-E354) and nearby amino acid residues (YΩ2, D1-H1190, D1-D61, D1-H337, CP43-R357, D1-N181, D2-K2317), and 17 surrounding water molecules including four water ligands (W1–W4), while other atoms were assigned to the MM region. D1-H337 was assumed to have a protonated cation form58 and W2 was fully protonated H2O3. Geometry optimization and normal mode analysis of the QM region were performed using an unrestricted DFT method with the B3LYP functional and the basis sets of LANL2DZ and 6-31 G(d) for metal atoms and other atoms, respectively. In geometry optimization, the coordinates of the QM region were fully relaxed, while those of the MM region were fixed. The oxidation states of the Mn ions were assumed to be III1V1 and III1V1 in high spin states (15et and 14et) in the S1 and S2 states, respectively. The S0 state was assumed to have an open cubane conformation with oxidized Mn4(IV). In calculation of OEC assumed for each normal mode and all bands in this region were co-added21,54. Calculated vibrational frequencies were scaled with a scaling factor of 0.9628 to match the major negative peak of the simulated S2/S1 spectrum of unlabeled OEC to the experimental peak at 1399 cm−1.

**Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data supporting the findings in this study are available within the manuscript and the Supplementary Information file. The sequence of cDNA of the psbA2 mRNA of D1-D170 was deposited in the DNA Data Bank of Japan under accession number LC717798. The coordinates of the PSIII complex used in QM/MM calculations were obtained from PDB ID: 4UB6. Source data are provided with this paper.

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Author contributions

T.N. designed the study. R.N. constructed the site-directed mutants and Y.S. isolated a histidine-tolerant strain. Y.S., T.M., and T.K. prepared samples and performed O2 evolution and FTIR analyses. T.S. and N.D. performed LC-MS analysis. T.N. performed QM/MM calculations. T.N. wrote the first draft, and Y.S., T.S., T.M., T.K., R.N., N.D., and T.N. completed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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