Supporting Information

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SI Discussion

Factors Affecting the Midpoint Redox Potential (E_m) of Q_A/Q_A^-. It has been shown that $E_m(Q_A/Q_A^-)$ is affected by several factors in higher plants and green algae; Mn depletion (1) and herbicide binding (2) are representative examples. In this study, we have shown that the same effects were reproduced in samples isolated from cyanobacteria. Under the control condition, we estimated the $E_m(Q_A/Q_A^-)$ in *Synechocystis* to be approximately −142 mV (Fig. 1*B*), which is consistent with a recent report on *Chlamydomonas reinhardtii* (−171 mV) and spinach (−162 mV) (3). Collective data have indicated a recent consensus of the $E_m(Q_A/Q_A^-)$ of approximately −150 mV. However, it is substantially different from the value of −84 ± 16 mV reported previously (1). In the previous report, the SD of 16 mV for the determination of $E_m(Q_A/Q_A^-)$ value seems to be rather large, especially compared with our measurements (1). Moreover, one of these authors had earlier obtained a value of −120 mV (error range unknown) by almost the same technique, except for the use of a mediator, for which they used methylviologen (4). In light of the last case, the value of −120 mV for Q_A of spinach might be the counterpart to our value (−146 mV); some other reasons for the discrepancy might be intactness and methodology of isolation of photosystem (PS) II particles from spinach. In addition, many studies (more than 35) on the redox potential of the Q_A have been reported, and the values range from −340 mV to +120 mV (1, 2). However, the origins of such a discrepancy is not clear for the moment.

Additionally, we showed that the Mn depletion–induced shift of the potential was ∼150 mV in *Synechocystis* (Figs. 1*B* and 3 and Table 1), and this value is similar to a previous report that included spinach, even though the absolute values under the control condition were different (1). Fufezan et al. determined that the $E_m(Q_A/Q_A^-)$ in PS II complexes from a cyanobacterium, *Thermosynechococcus elongatus*, is +84 mV, which is ∼160 mV greater than that of the active form of Q_A in spinach PS II (5). The authors argued that this variance could reflect a difference in species or Mn depletion during titration. The reproducibility of the Mn depletion–induced changes was surprisingly high among several experiments (140–150 mV). In this sense, a common mechanism might be responsible for this phenomenon.

In early studies, it has been shown that the effect of herbicides on the redox potential of Q_A/Q_A^- depends on the molecular species of herbicides used; according to Krieger and Rutherford (2), the redox potential of Q_A is down-shifted in the presence of bromoxynil, a phenolic herbicide. In contrast, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) induces an up-shift of the potentials (2). In the present study, we reproducibly observed that DCMU induced an up-shift of the potentials of Q_A^- in spinach, *Synechocystis*, and *Acaryochloris marina* of ∼30–50 mV (Table 1), even though the magnitude of the shift was slightly different among the samples. This DCMU effect was not abolished after Mn depletion. In this sense, this potential shift was independent of the overall changes in the structure of PS II; rather, it depended on a local effect. To further investigate this effect, it might be useful to construct a mutant whose $E_m(Q_A/Q_A^-)$ is insensitive to DCMU treatment for the analysis of energetics in PS II. Additionally, as theoretically indicated by Ishikita et al., the redox potential of the special pair component is significantly affected not only by the nearby amino acids but also by the protein backbone (6). This characteristic may be extended to all electron transfer components and should be a subject of future study.

The charge recombination rates between P^+ and pheophytin (Phe) a^- at 77 K observed in intact cells were also very similar between *Synechocystis* (7) and *A. marina* (8), supporting the idea of a consistent architecture of the primary reaction systems between the two organisms. A difference in the energetics, which was caused by a difference in the energy gain by the special pair, was found only in the absolute potential values. The adjustment of potential values in *A. marina* with a magnitude of <100 mV would likely be realized through the modification of hydrogen bonds, the relative position of prosthetic groups, and the protein backbone (6).

SI Materials and Methods

Preparation of the Mn-Depleted PS II. ***Tris treatment.*** Spinach PS II particles in 0.1 mg of Chl·mL^{-1} were suspended in 1 M Tris·HCl (pH 8.0) plus 0.5 M $MgCl_2$ and incubated for 45 min in darkness on ice (9). More than 95% of the Mn was removed by this treatment. This treatment was followed by two washes of the particles with 40 mM Mes/NaOH (pH 6.5), 20 mM NaCl, 0.4 M sucrose, and 0.5 mM EDTA and another two washes with the same buffer without EDTA. The particles were then resuspended in the titration buffer [50 mM Mes/NaOH (pH 7.0), 10 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$, 0.04% β-D-dodecyl maltoside, and 1 M betaine].

NH_2OH treatment. Mn depletion by NH_2OH treatment was carried out by incubation of the PS II particles (spinach; 0.1 mg of Chl·mL^{-1}) in 40 mM Mes/NaOH (pH 6.5), 20 mM NaCl, 0.4 M sucrose, 1 mM EDTA, and 10 mM NH_2OH at 4 °C for 60 min in darkness, followed by two washes in the same buffer without EDTA or NH_2OH. The Mn-depleted PS II particles were sedimented by centrifugation at 20,000 × *g* for 20 min and then resuspended in the titration buffer [50 mM Mes/NaOH (pH 7.0), 10 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$, 0.04% β-D-dodecyl maltoside, and 1 M betaine].

2-(Cyclohexylamino)ethanesulfonic acid (CHES) treatment. PS II (spinach) particles, at a concentration of 0.1 mg of Chl·mL^{-1}, were incubated in 20 mM CHES/NaOH buffer (pH 9.4) in the presence of 200 mM $MgCl_2$ for 3–4 min at room temperature, followed by dilution up to 10- to 15-fold with 50 mM Mes/NaOH buffer (pH 6.5) to stop the reaction (10–13). The membranes were washed once with 40 mM Mes/NaOH (pH 6.5), 20 mM NaCl, 0.4 M sucrose, and 1 mM EDTA and twice in the EDTA-free buffer and then resuspended in the titration buffer [50 mM Mes (pH 7.0), 10 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$, 0.04% β-D-dodecyl maltoside, and 1 M betaine].

PS II Core Complexes. ***Tris treatment.*** A complete (>95%) removal of Mn from the PS II core complexes from *Synechocystis* sp. PCC 6803 (0.1 mg of Chl·mL^{-1}) was achieved by using 1 M Tris·HCl (pH 8.5) plus 0.5 M $MgCl_2$ with incubation for 30 min in darkness on ice (14). This treatment was followed by washing twice with 40 mM Mes/NaOH (pH 6.5), 10 mM $MgCl_2$, 10% PEG6000, and 1 mM EDTA and twice in the EDTA-free buffer. The complexes were then resuspended in the titration buffer [50 mM Mes/NaOH (pH 7.0), 10 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$, 0.04% β-D-dodecyl maltoside, and 1 M betaine].

NH_2OH treatment. The NH_2OH treatment was performed as follows. Intact, O_2-evolving PS II core complexes from *Synechocystis* sp. PCC 6803 were incubated with 0.1 mg of Chl·mL^{-1} for 30 min at 4 °C in darkness in 40 mM Mes (pH 6.5), 10 mM NaCl, 10 mM $MgCl_2$, 5 mM $CaCl_2$, and 25% glycerol in the presence of 10 mM NH_2OH and 1 mM EDTA, as described (15). This treatment was followed by two washes in the same buffer without EDTA or NH_2OH. Before centrifugation, we added 10% PEG6000 to the solution. The pellet was resuspended in the titration buffer

[50 mM Mes/NaOH (pH 7.0), 10 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$, 0.04% β-D-dodecyl maltoside, and 1 M betaine].

CHES treatment. PS II particles, at a concentration of 0.1 mg of Chl·mL^{-1}, were incubated in 20 mM CHES/NaOH buffer (pH 9.4) in the presence of 200 mM $MgCl_2$ for 2–3 min at room temperature, followed by dilution with 50 mM Mes/NaOH buffer (pH 6.5) to stop the reaction. The membranes were washed once with 40 mM Mes/NaOH (pH 6.5), 20 mM NaCl, and 0.4 M sucrose in the presence of 1 mM EDTA and twice in the EDTA-free buffer and then resuspended in the titration buffer [50 mM Mes (pH 7.0), 10 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$, 0.04% β-D-dodecyl maltoside, and 1 M betaine]. Before centrifugation, we added 10% PEG6000 to the buffer.

These treatments of the PS II particles or core complexes were devoid of the three extrinsic proteins, as determined by SDS/PAGE, and exhibited no residual O_2 evolution activity. The residual Mn content in the PS II samples was estimated to be less than 0.1–0.2 Mn molecules per reaction center.

Reconstitution of the Mn-Depleted PS II Complexes. The restoration of O_2 evolution in the Mn-depleted PS II complexes was performed as previously described (10–13, 16) in an assay buffer containing 50 mM Mes/NaOH (pH 6.5), 35 mM NaCl, 5 mM $CaCl_2$, and 0.4 M sucrose in the presence of 2 mM $K_3Fe(CN)_6$ as the terminal electron acceptor. The samples, at a concentration of 0.1 mg of Chl·mL^{-1}, were incubated in a glass tube for 10 min at 4 °C in the dark in the presence of $MnCl_2$ (200 μM) and $NaHCO_3$ (5 mM). Photo-activation was then conducted by illumination with white light, using an intensity of ~40–50 μE $m^{-2}·s^{-1}$ for 20 min. The activity of PS II of these samples was measured by monitoring the photosynthetic evolution of oxygen at 25 °C in the presence of 2 mM $K_3Fe(CN)_6$ as electron acceptor with a Clark-type oxygen electrode (Fig. S3) (17).

Phe Titration. We adopted a titration procedure previously described (17). The redox titration was performed under anaerobic conditions (under argon) in a reaction mixture under physiological conditions (pH 7.0 at 25 °C) with a low concentration of Chl (~8 μg of Chl·mL^{-1}). The intensity of the measuring light was 4 μmol photons $m^{-2}·s^{-1}$. The light-induced difference spectra were obtained by subtracting the spectra of illuminated samples from the spectra of control (dark) samples. The samples were illuminated by saturated light for only 15 s to avoid possible photo-induced damage to the samples.

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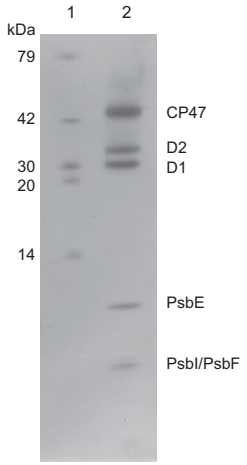

Fig. S1. SDS/PAGE patterns of the PS II complexes of *A. marina* used in this study. Lane 1, molecular markers; lane 2, PS II complex of *A. marina*. The polypeptide assignments are indicated on the right.

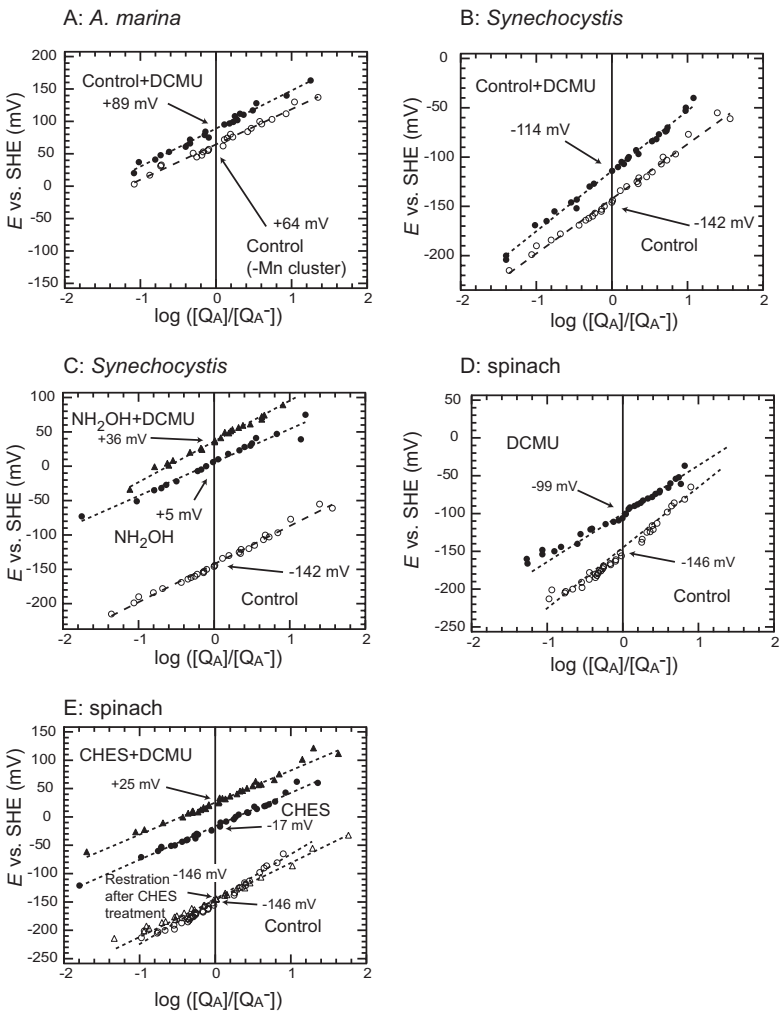

Fig. S2. The redox titration of Q_A/Q_A^- in PS II complexes *A. marina*, with or without DCMU (*A*); *Synechocystis*, with or without DCMU (*B*); *Synechocystis*, control, after NH_2OH treatment, after NH_2OH treatment followed by the addition of DCMU (*C*); spinach, with or without DCMU (*D*); and spinach, control, after CHES treatment, after CHES treatment followed by the addition of DCMU, and restoration after CHES treatment (*E*).

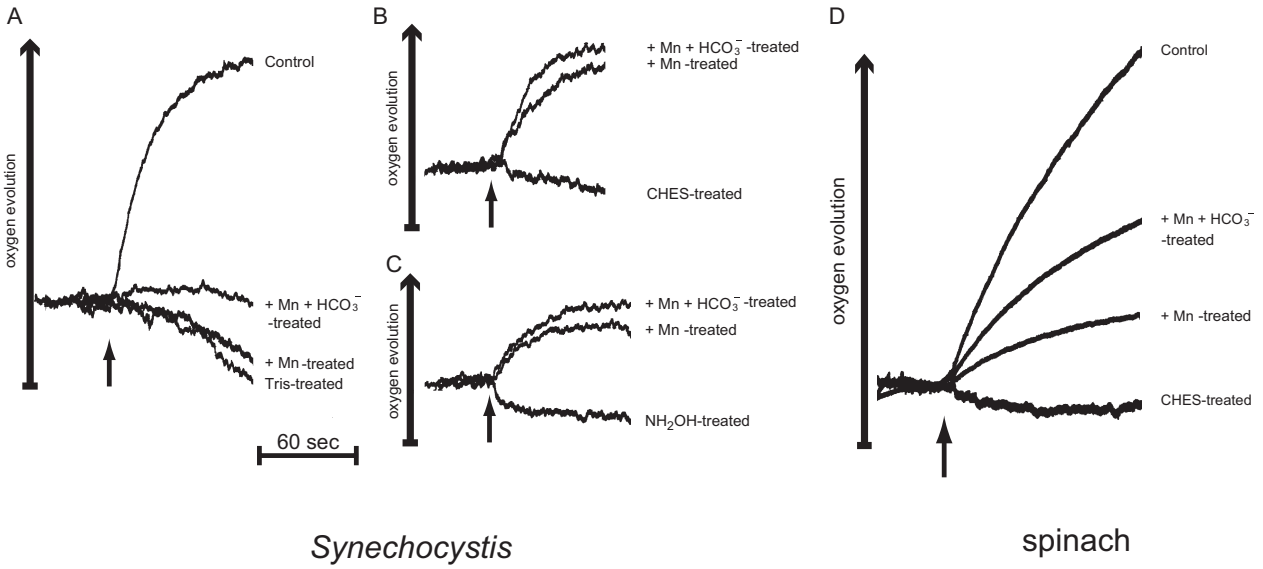

Fig. S3. Oxygen-evolving traces and the effect of Mn depletion and restoration on PS II complexes isolated from *Synechocystis* and spinach. The PS II core complexes from *Synechocystis* (*A*–*C*) and BBY particles from spinach (*D*) were depleted of Mn by Tris (*A*), CHES (*B* and *D*), or NH_2OH (*C*) treatment and restored by addition of 200 μM $MnCl_2$ or 200 μM $MnCl_2$ with 5 mM $NaHCO_3$. Arrows indicate the time point at which the light was switched on. Photo-activation of the Mn cluster was achieved by the addition of reagents indicated (*Materials and Methods*). For more details of restoration of the Mn-depleted PS II complexes, see *SI Materials and Methods*.