Supporting Information

Allakhverdiev et al. 10.1073/pnas.0913460107

SI Materials and Methods

Redox titration was performed under anaerobic conditions (under argon) in a reaction mixture [5 mL; ~8 μg chlorophyll (Chl)·mL^{-1}] containing 50 mM Mes (pH 7.0), 10 mM NaCl, 2 mM $MgCl_2$, 5 mM $CaCl_2$, and 0.04% β-D-dodecyl maltoside in the presence of 1,1′-trimethylene-2,2′-dipyridinium dibromide (2 μM; E_{m7} = −552 mV), sodium dithionite (50–100 μM; E_{m7} = −530 mV), methyl viologen (2 μM; E_{m7} = −440 mV), benzyl viologen (2 μM; E_{m7} = −311 mV), or indigo disulfonate (2 μM; E_{m7} = −125 mV). For potentials lower than −550 mV, the concentrations of sodium dithionite, 1,1′-trimethylene-2,2′-dipyridinium dibromide, and indigo disulfonate were increased to 150–200 μM, 5 μM, and 5 μM, respectively, and neutral red (up to 20 μM; E_{m7} = −325 mV) was added gradually. The presence of these redox mediators produced an E_m = −628 mV and did not change the pH of the medium. However, to further lower the oxidation potential, we changed the pH of the medium from 7.0 to 7.3. At a low concentration of sodium dithionite, redox equilibrium was reached slowly. After titrations in the reducing direction, reoxidation was performed several times on the same samples. Oxidative titrations were performed by gradual addition of potassium ferricyanide (up to 100 μM; E_{m7} = 430 mV). Most titrations were performed from low to high potential, and the dark relaxation time interval between measurements at two different potentials was 10–15 min. Each sample was used for two to four different measurements of potential in the reducing or oxidizing direction for a total of at least 20 data points.

The light-induced absorption changes of pheophytin (Phe) *a* were monitored using a Hitachi U-0080D photodiode array spectrophotometer at 25 °C at different ambient redox potentials. Actinic light from a halogen lamp (150 W) was used for illumination through a glass fiber using a blue (Corning 4–96) or red filter (R-60; Toshiba), and the intensities of the actinic light were 900 and 1,600 μmol photons m^{-2}·s^{-1}, respectively. Additional red or blue filters were placed before the photodiode, as needed. The intensity of measuring light was 4 μmol photons m^{-2}·s^{-1}. The light-induced difference spectra were obtained by subtraction of the spectra of illuminated samples from the spectra of control (dark) samples. The intensity of the actinic light was saturated, and the sample was illuminated for 15 s to avoid a possible photoinhibition (1).

1. Nikitishena OV, Smolova TN, Khatypov RA, Shkuropatov AJ, Klimov VV (2002) A new pathway of photoinactivation of photosystem II: Irreversible photoreduction of pheophytin causes loss of photochemical activity of isolated D1/D2/Cyt *b*559 complex. *Biochemistry (Mosc)* 67:364–371.

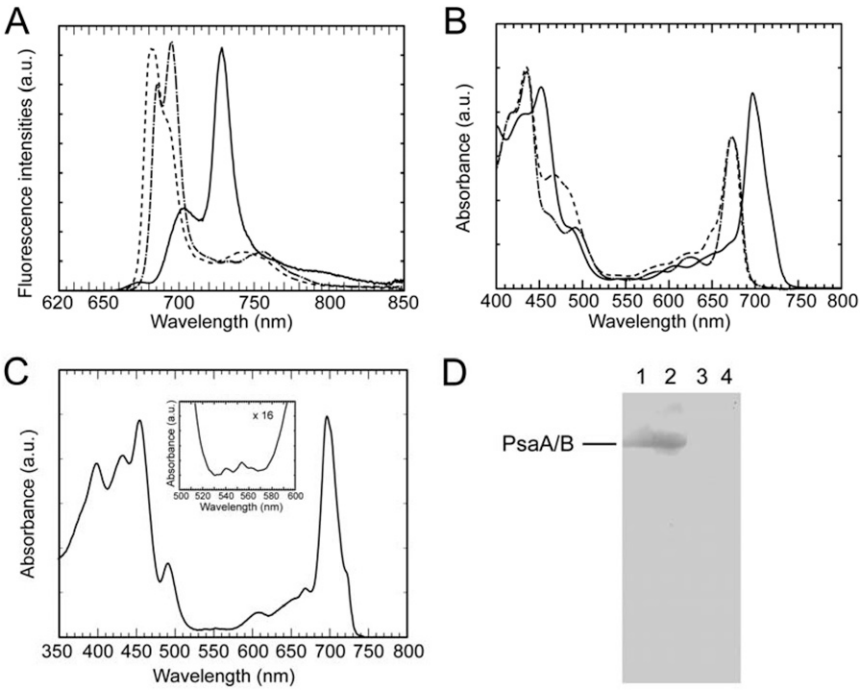

Fig. S1. Purity of samples was investigated by fluorescence and absorption spectra and by immunoblotting analysis. (*A*) Fluorescence spectra of PS II complexes at −196 °C excited at 435 nm, and (*B*) absorption spectra of photosystems (PS) II complexes at room temperature. *A* and *B* show PS II complexes from *Synechocystis* sp. PCC 6803 (dash-dot line), *Acaryochloris marina* (solid line), and spinach chloroplasts (broken line). (*C*) The absorption spectrum of PS II core complexes isolated from *A. marina* at −193 °C is shown. (*D*) PsaA/B subunits were identified by immunoblotting analysis. Lane 1, thylakoid membrane (*A. marina*); lane 2, thylakoid membrane (*Synechocystis*); lane 3, PS II complex (*A. marina*); lane 4, PS II complex (*Synechocystis*). *Synechocystis* and spinach samples did not show fluorescence band(s) from PS I components ($\lambda > 720$ nm). In the case of *A. marina*, PS I fluorescence was very weak in intact cells (1) and was detected at 732 nm in isolated complexes (2). A peak detected at 728 nm is typical of PS II fluorescence (3). Western blotting data confirmed the absence of PS I components. The absorption spectra of the samples showed peaks at 674, 697, and 673 nm for the PS II complexes isolated from *Synechocystis*, *A. marina*, and spinach chloroplasts, respectively, at 25 °C. In the PS II core complexes of *A. marina*, the number of Chl *d* and Chl *a* per two Phe *a* molecules was estimated to be 29.6 and 1.9, respectively. These values are dramatically lower than the previous report (3). This difference arose from the removal of chlorophyll protein (CP) 43′ as confirmed by the SDS/PAGE profile (Fig. 1). The absorption spectrum of *A. marina* PS II at −193 °C showed the presence of a short wavelength component at 672 nm, which was probably Chl *a*, and it was detected by a decrease in antenna pigments.

1. Mimuro M, Hirayama K, Uezono K, Miyashita H, Miyachi S (2000) Uphill energy transfer in a chlorophyll *d*-dominating oxygenic photosynthetic prokaryote, *Acaryochloris marina*. *Biochim Biophys Acta* 1456:27–34.
2. Tomo T, et al. (2008) Characterization of highly purified photosystem I complexes from the chlorophyll *d*-dominated cyanobacterium *Acaryochloris marina* MBIC 11017. *J Biol Chem* 283:18198–18209.
3. Tomo T, et al. (2007) Identification of the special pair of photosystem II in the chlorophyll *d*-dominated cyanobacterium. *Proc Natl Acad Sci USA* 104:7283–7288.

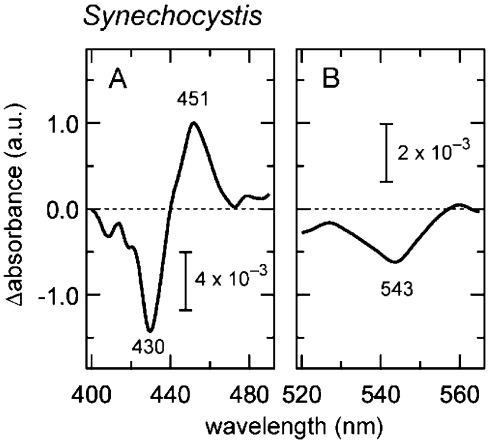

Fig. S2. A light-minus-dark difference absorption spectrum of *Synechocystis* PS II in the blue regions (*A*) and the Qx region (*B*).

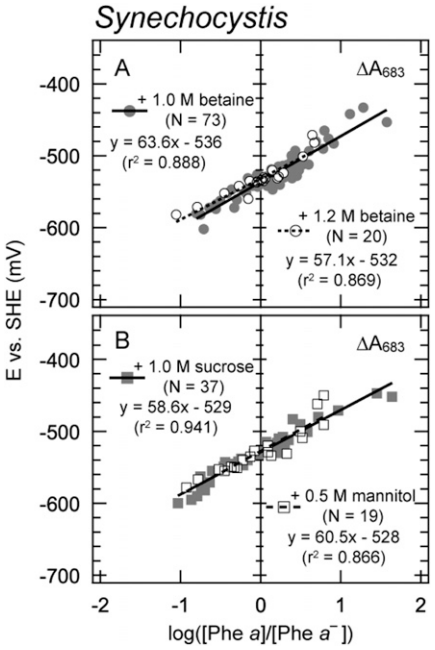

Fig. S3. Effect of stabilizers on the redox potentials of Phe *a* in PS II complexes isolated from *Synechocystis*. The effect of betaine (1.0 M and 1.2 M), sucrose (1.0 M), and mannitol (0.5 M) on the redox potentials of Phe *a* was shown by the titration curves. Detection wavelengths are indicated in the upper right of each panel. *N*, number of data points; r^2, correlation coefficient.

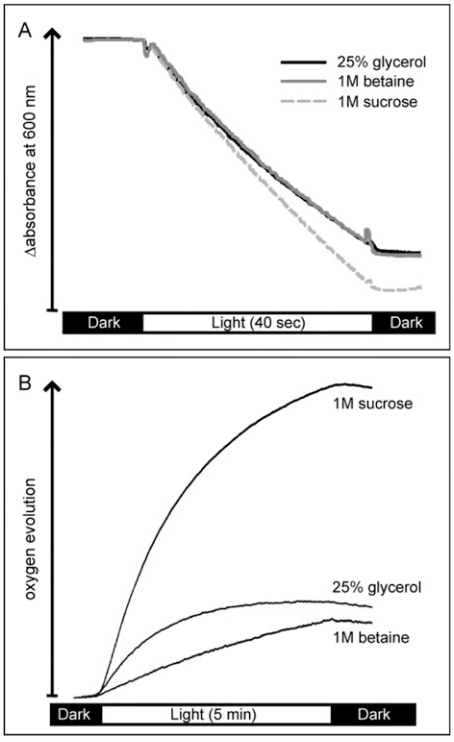

Fig. S4. Effect on betaine on dichlorophenol indophenol photoreduction in the presence of diphenyl carbazide (DPC–DCIP) (*A*) and oxygen evolution (*B*) in PS II complexes isolated from *Synechocystis*. DPC–DCIP photoreduction was monitored by the changes in absorbance at 600 nm. Betaine (1.0 M) and 25% glycerol gave identical traces, indicating that both reagents did not induce any acceleration in the rate of DPC–DCIP photoreduction, but sucrose enhanced the rate by ~10%. Sucrose (1.0 M) enhanced the initial rate of O_2 evolution by ~250%; however, betaine (1.0 M) did not enhance the initial rate. In contrast, the rate of O_2 evolution remained constant for up to 5 min of illumination in the presence of betaine, but the initial rate was saturated within 2 min of illumination in the presence of sucrose and glycerol (25%). These results indicate that the sites at which betaine and sucrose interact with PS II may differ.