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Alterations in photosynthetic pigments and amino acid composition of D1 protein change energy distribution in photosystem II

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ABSTRACT

The marine cyanobacterium *Prochlorococcus marinus* accumulates divinyl chlorophylls instead of monovinyl chlorophylls to harvest light energy. As well as this difference in its chromophore composition, some amino acid residues in its photosystem II D1 protein were different from the conserved amino acid residues in other photosynthetic organisms. We examined PSII complexes isolated from mutants of *Synechocystis* sp. PCC 6803, in which chromophore and D1 protein were altered (Hisashi Ito and Ayumi Tanaka, 2011) to clarify the effects of chromophores/D1 protein composition on the excitation energy distribution. We prepared the mutants accumulating divinyl chlorophyll (DV mutant). The amino acid residues of V205 and G282 in the D1 protein were substituted with M205 and C282 in the DV mutant to mimic *Prochlorococcus* D1 protein (DV-V205M/G282C mutant). Isolated PSII complexes were analyzed by time-resolved fluorescence spectroscopy. Energy transfer in CP47 was interrupted in PSII containing divinyl chlorophylls. The V205M/G282C mutation did not recover the energy transfer pathway in CP47, instead, the mutation allowed the excitation energy transfer from CP43 to CP47, which neighbors in the PSII dimer. Mutual orientation of the subcomplexes of PSII might be affected by the substitution. The changes of the energy transfer pathways would reduce energy transfer from antennae to the PSII reaction center, and allow *Prochlorococcus* to acquire light tolerance.

1. Introduction

Most oxygenic photosynthetic organisms contain 3-vinyl chlorophyll (monovinyl chlorophyll, MV-Chl). However, the marine picophytoplankton *Prochlorococcus* contains 3,8-divinyl chlorophyll (DV-Chl) instead of MV-Chl [1]. The Soret band of DV-Chls shifts about 10 nm towards the red compared with that of MV-Chls [2,3]. Therefore, *Prochlorococcus* has the advantage of absorbing blue light in deep water layers [4]. Recently, we reported on the photochemical properties of a *Synechocystis* sp. PCC 6803 (hereafter referred to as *Synechocystis*) mutant that exhibits a predominant replacement of MV-Chls with DV-Chls [3]. This mutant was rapidly bleached under high-light conditions, and the photosystem II (PSII) subunits (i.e., CP43 and PsbO) were dissociated from PSII by high-light treatment. Another mutant of *Arabidopsis thaliana* that contains DV-Chls also rapidly died under high-light conditions [4]. In both of these mutants, PSII fluorescence showed a red peak shift compared with that of the wild-type, and the delayed fluorescence from PS II was suppressed in the 680–685 nm wavelength region [5–7]. The delayed fluorescence reflects excitation energy distribution around PSII reaction center (PSII-RC) [8]. Therefore, replacement of MV-Chls by DV-Chls affects energy transfer pathways in PSII.

Prochlorococcus species have two altered amino acids in the PSII D1 subunit compared with those in other oxygenic photosynthetic organisms [9]. One of these amino acids, V205M, is located near P680, and the other, G282C, is located near the surface between the D1 and CP43 subunits. These amino acid alterations improve high-light tolerance, where the *Synechocystis* mutant accumulating DV-Chl (DV mutant) died under medium-light condition (250 μmol m^{-2} s^{-1}) but the *Synechocystis* mutant accumulating DV-Chl with two altered amino acids in D1 subunit (DV-V205M/G282C mutant) grew well [9]. Therefore, it was expected that optimization of amino acid sequences might adjust energy transfer pathways to improve high-light tolerance in PSII complexes containing DV-Chl.

In the present study, we analyzed time-resolved fluorescence spectra (TRFS) of PSII isolated from the DV mutant and the DV-V205M/G282C mutant. Fluorescence decay-associated spectra (FDAS) and delayed fluorescence spectra revealed that the energy transfer pathway

Abbreviations: PSII, photosystem II; RC, reaction center; MV-Chl, chlorophyll *a*; DV-Chl, 3,8-divinyl chlorophyll *a*; FDAS, fluorescence decay-associated spectrum; TRFS, time-resolved fluorescence spectra

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in CP47 was depressed in the DV mutant, and excitation energy was accumulated around PSII-RC. The accumulated energy was diverted to CP47 in the DV-V205M/G282C mutant without recovery of the depressed pathway, and PSII-RC received less excitation energy. The change of energy transfer pathway may relate to improve of the high-light tolerance in the DV-V205M/G282C mutant.

2. Materials and methods

2.1. Sample preparations

We isolated the PSII dimer complexes from following three mutants [5,9]. In brief, all *Synechocystis* transformants were prepared from *Synechocystis* engineered to express a 6× histidine-tag at CP47 and were prepared by the disruption of *psbA1* and *psbA3* (MV mutant). In addition, the *slr1923* locus encoding 3,8-divinyl chlorophyllide 8-vinyl reductase was disrupted (DV mutant). Furthermore, two amino acid residues of D1 protein, V205 and G282, are substituted with M205 and C282 (DV-V205M/G282C mutant). Here after, we call the isolated PSII samples as MV, DV, and DV-V205M/G282C samples. The accumulation levels of CP43 and CP47 were same in the samples (Fig. S1). Generation of mutants and growth conditions of the mutants are described elsewhere [9].

2.2. Spectroscopic measurements

Steady-state absorption and fluorescence spectra at 77 K were measured using spectrometers (JASCO V-650, JASCO FP-6600). TRFS were measured by the time-correlated single-photon counting method at 77 K [10]. The excitation wavelength was set at 425 nm and the repetition rate of the pulse trains was 2.9 MHz, which did not interfere with measurements up to 100 ns (24.4 ps/channel × 4096 channels). To improve time resolution, time-resolved fluorescence was also measured up to 10 ns (2.4 ps/channel × 4096 channels). Spectral data were measured at 1-nm intervals. The decays were fitted using convolution and simulation method (Fig. S2). A homemade program controlled by Mathematica (ver. 7, Wolfram Research) was used to fit decay curves. We set two constrains in fitting procedure, the lower limit of lifetime value was set to 2.4 ps, and the upper limit of lifetime value was set to 90 ns. Instrumental function and free exponential components were used to simulate fitting curves, and the determined exponential components were used to construct deconvoluted decay curves. The deconvoluted decay curves were imported to Igor (ver. 6, WaveMetrics) to perform global analyses. Following a global analysis of the fluorescence kinetics, FDAS were constructed [10]. Based on change of chi-squares and differences between decay curves and fitting curves (Fig. S3), we chose results of four components analyses (~50 ps, ~200 ps, 1–6 ns, and ~35 ns). The 1–6 ns components showed similar spectral shapes and were combined to one component with its mean lifetime (~4.5 ns).

3. Results and discussion

3.1. Steady-state measurements

Fig. 1 shows absorption spectra of PSII complexes at 77 K. In the DV and DV-V205M/G282C samples, Soret bands were shifted to the red by 7 nm compared with those in the MV sample. This shift helps to absorb blue light. On the contrary, the Qy-bands were located at almost the same wavelength in all samples, which reflects similar S_1 energy levels between MV- and DV-Chl *a*. Since the S_1 state of Chl *a* is responsible for energy transfers, the similarity of the S_1 energy levels may help to keep energy transfer pathways in PSII complex even if the exchange of chromophores from MV-Chl *a* to DV-Chl *a* occurs. However, we should note here that energy transfer pathways are affected not only by energy levels but also by orientations of chromophores. The fluorescence spectra clearly reflected energy transfer dynamics.

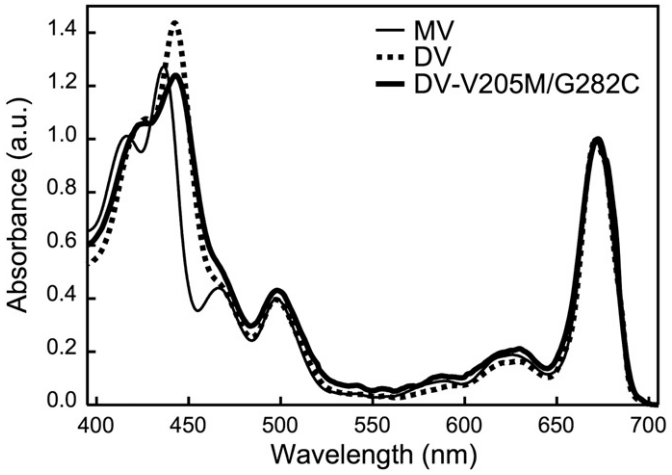


Fig. 1. Absorption spectra of isolated PSII complexes at 77 K. Spectra were normalized at 680 nm. MV sample, thin solid line; DV sample, thick dashed line; DV-V205M/G282C sample, thick solid line.

Fig. 2 shows fluorescence emission spectra of the PSII complex at 77 K. Two peaks at 686 nm and 694 nm were observed in the MV sample (Fig. 2a), which correspond to CP43 and CP47, respectively. In the DV sample (Fig. 2b), two peaks were observed at 688 nm and 694 nm. As revealed by previous time-resolved fluorescence study [11], the PSII fluorescence exhibits a peak at shorter wavelength just after excitation (682–686 nm) and the peak shift to the red with time (695 nm), showing energy transfer in PSII. The 688-nm peak might indicate partial disruption of energy transfer from Chl_{688} to Chl_{694} in CP47. The relative intensity of the 694-nm peak was lower in the DV sample than in the MV sample, which also reflects reduced

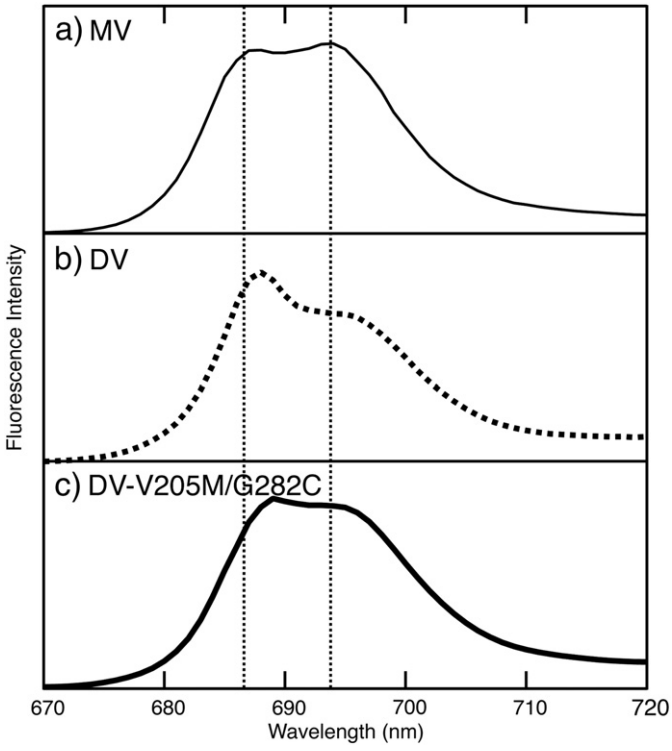


Fig. 2. Fluorescence spectra of isolated PSII complexes at 77 K. Excitation wavelength, 440 nm. MV sample, thin solid line; DV sample, thick dashed line; DV-V205M/G282C sample, thick solid line. Vertical lines indicate wavelengths of fluorescence maxima in MV.

efficiency of energy transfer to Chl_{694} [5]. In the DV-V205M/G282C sample, there was an increase in the relative intensity of the peak at 694 nm. Two candidates are conceivable to explain this recovery in the 694-nm peak intensity: improvement in energy transfer to Chl_{694} and construction of a new energy transfer pathway to Chl_{694}. The 688-nm peak was still observed in the DV-V205M/G282C sample, indicating the energy transfer pathway from Chl_{688} to Chl_{694} might keep being disrupted in the DV-V205M/G282C sample. Therefore, it seems that a new energy transport pathway to Chl_{694} was constructed in the DV-V205M/G282C sample.

3.2. Time-resolved measurements

To examine changes in the transfer pathway, we measured TRFS of the PSII complexes at 77 K. We applied the FDAS method to resolve TRFS into spectral forms with the same rise and decay profiles. Fig. 3 shows FDAS of the PSII complexes at 77 K. Positive and negative amplitudes correspond to fluorescence decay and rise components, respectively. Four lifetime components (tens of ps, hundreds of ps, a few ns, and longer than 10 ns) were necessary to fit fluorescence rise and decay profiles. For further analysis, we resolved each FDAS into three Chl-fluorescence components that consist of a main peak and a long-wavelength tail. The highest-energy component showed a peak at 686 nm (F686), and two additional components at 688 and 694 nm were resolved (F688, F694). These three peaks were also observed in raw data (Fig. S4). Raszewski et al. reported the site energies and corresponding absorption wavelengths of Chls in the PSII complex [12]. Accordingly, we assigned F688 and F694 to Chls in CP47 (the former to Chl_{687} and Chl_{688}, and the latter to Chl_{694}, Scheme 1), assuming a 7-nm Stokes shift. F686 originated from Chl_{686} in CP43. These assignments were consistent with previous reports about *Synechocystis* mutants of CP43 and CP47 [13,14] and spectroscopic properties of isolated PSII complexes [11,15–18]. Other chlorophylls without annotations have higher site energies (671–683 nm), and they could also contribute to the blue edge of F686.

Fig. 3a shows the FDAS of the MV sample. In the 75-ps component, F686 showed a positive amplitude, whereas F688 and F694 showed negative amplitudes, which reflect fast energy transfer to Chl_{687}, Chl_{688}, and Chl_{694}. In the 200-ps component, F688 and F694 showed a positive and a negative amplitude, respectively, indicating slow energy transfer from Chl_{687} and/or Chl_{688} to Chl_{694}. Total trapping also contributes to the positive amplitude.

Fig. 3b shows the FDAS of the DV sample. In the 45-ps component, F686 and F688 showed a positive and a negative peak, respectively, but the amplitude of F694 was negligibly small. Chl_{687} and/or Chl_{688} accepted energy from Chl_{686}; however, Chl_{694} was no longer able to accept energy quickly in the DV sample. Absence of F686 in the 230-ps component might reflect slight increase of efficiency of energy transfer from Chl_{686}. In the 230-ps component, F688 and F694 showed a positive and a negative amplitude, respectively, indicating that the slow energy transfer pathway from Chl_{687} and/or Chl_{688} to Chl_{694} was still available. Scheme 1 shows two possible pathways to Chl_{694}. Our results suggest that at least one of the two pathways may be perturbed in the DV sample (Scheme 1, blue crosses).

Fig. 3c shows the FDAS of the DV-V205M/G282C sample. In the 45-ps component, F686 showed a positive amplitude, whereas F688 and F694 showed negative amplitudes, reflecting fast energy transfer to Chl_{687}, Chl_{688}, and Chl_{694}. The amplitude of F694 was twice as large as that in the MV sample, which indicates improvement and/or construction of a new fast energy transfer pathway to Chl_{694}. F694 did not show a negative amplitude in the 200-ps component, indicating that most energy was transferred to Chl_{694} via a fast pathway(s) in the DV-V205M/G282C sample.

The 4.2–4.5 ns components reflect excitation energy distribution in PSII after energy transfer processes caused by laser excitation [16,19]. Because Chl content in the PSII-RC is much smaller than

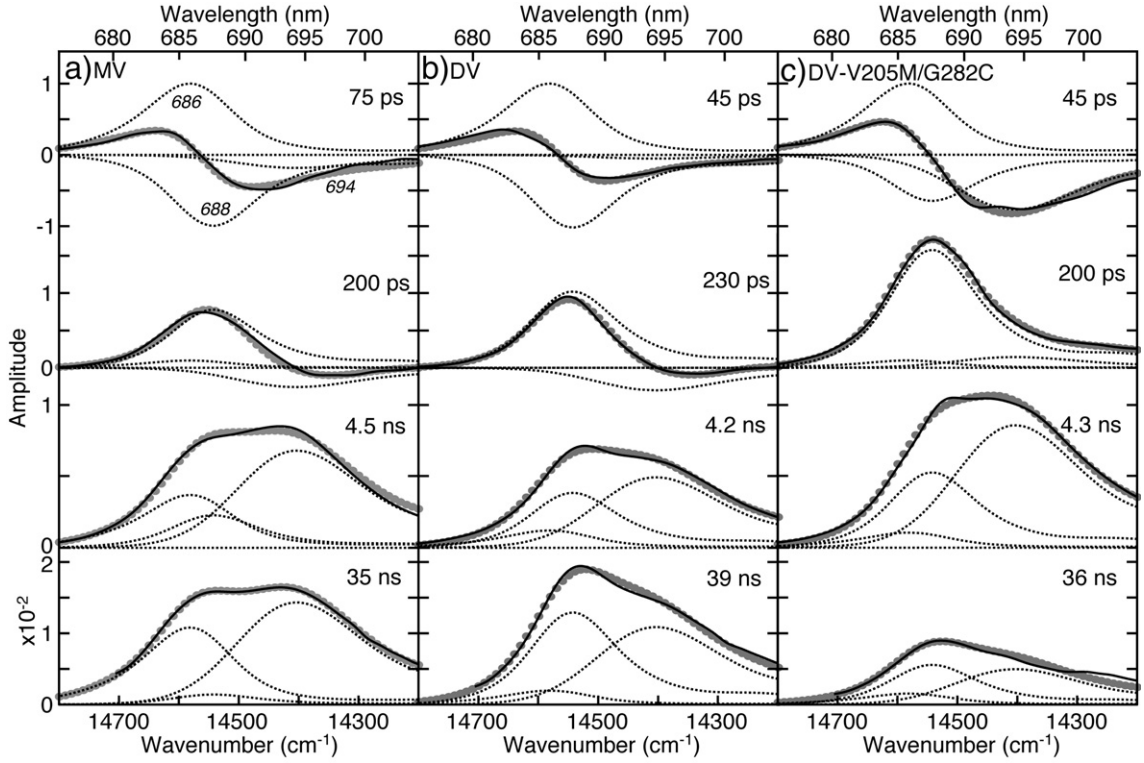
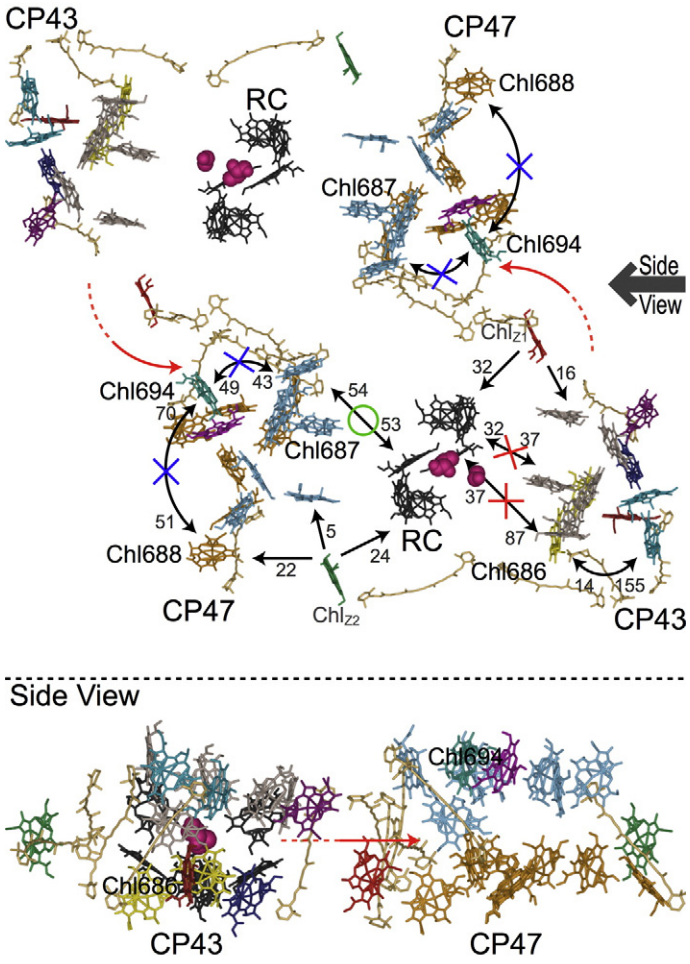


Fig. 3. FDAS of isolated PSII complexes at 77 K (solid line). Spectral components and their summation are shown as dotted lines and gray circles, respectively. (a) MV sample, (b) DV sample, (c) DV-V205M/G282C sample. All curves were normalized to the maximum intensity of F686 in the shortest lifetime components in each samples (MV, 75 ps; DV, 45 ps; DV-V205M/G282C, 45 ps).



Scheme 1. Change of energy transfer pathways in PSII. Crystal structure of PSII was obtained from protein databank (PDB ID: 3ARC) and is colored as shown in Fig. 10 of reference [12]. Chls with the same color belong to the same exciton domain [12]. Arrows represent energy transfer pathways, where disorder-averaged inverse rate constants in units of picoseconds are also shown in italic type [12]. Pink spheres show positions of altered amino acid residues in D1 protein (V205M and G282C). DV and DV-V205M/G282C samples showed decreases in efficiency of energy transfer pathways from Chl_{687} and/or Chl_{688} to Chl_{694} (blue crosses). In addition, DV-V205M/G282C sample showed decreased energy transfer from Chl_{686} to PSII-RC (red crosses) and increased energy transfer via another pathway to Chl_{694} (red arrows).

that in the PSII core antenna, the laser pulse was mostly absorbed by antenna Chls in CP43 and CP47; therefore, the 4.2–4.5 ns components indicate energy trap(s) in PSII core antenna. In all samples, the amplitude of F694 was larger than those of F686 and F688, indicating energy transfer to Chl_{694}. However, the highest amplitude of F694 was found in the DV-V205M/G282C sample, and the lowest was in the DV sample. Under antenna-excitation condition, the DV-V205M/G282C sample showed the most efficient energy transfer to Chl_{694}.

The delayed fluorescence spectra (35–39 ns components) reflect energy distribution after the charge recombination at PSII-RC [11]. In other words, we can view the delayed fluorescence components as fluorescence spectra with direct excitation of PSII-RC [8]. F686 and F694 were the predominant components in the MV sample, whereas F688 showed the largest amplitude in the DV and DV-V205M/G282C samples, indicating that the energy transfer pathways between PSII-RC and Chl_{694} were partially blocked in both samples. *Prochlorococcus* also shows delayed fluorescence maxima around 688 nm [20]. This suggests that the block may be caused by the substitution of MV-Chl by DV-Chl in PSII-RC. The change will most probably be induced through steric hindrance and/or interactions between Chl molecules and peptide moieties [7]. The pathways between PSII-RC and Chl_{694} were partially blocked in both the DV and DV-V205M/G282C samples. However, as discussed above, fast energy transfer to Chl_{694} was observed in the DV-V205M/G282C sample (Fig. 3c; 45-ps component). These results suggest that V205M and G282C did not recover the pathway between PSII-RC and Chl_{694} (blue crosses in Scheme 1) but generated a new pathway(s) to Chl_{694}. In other words, the efficient energy transfer to Chl_{694} was achieved as a result of construction of a new, fast energy transfer pathway in the DV-V205M/G282C sample. Furthermore, the delayed fluorescence amplitude in the DV-V205M/G282C sample (Fig. 3c; 36-ns component) was only half of those in the MV and DV samples, indicating that the energy transfer from the antennae to PSII-RC was blocked by approximately 50% in the DV-V205M/G282C sample.

Scheme 1 illustrates changes in energy transfer pathways in PSII. When MV-Chl was replaced with DV-Chl (the DV sample), the pathway(s) from Chl_{687} and/or Chl_{688} to Chl_{694} may have been perturbed in CP47 (Scheme 1, blue crosses). If this occurs, then Chl_{694} cannot accept energy quickly (Fig. 3b; 45-ps component) and Chl_{688} accumulates energy transferred from PSII-RC (Fig. 3b; 39-ns component). Even though the amino acids of the D1 protein were altered to match those of the D1 protein in *Prochlorococcus* (Scheme 1, pink spheres), Chl_{688} still accumulated energy from PSII-RC in the DV-V205M/G282C sample

(Fig. 3c; 36-ns component), which suggests that the pathway(s) marked with blue crosses remained slow in the DV-V205M/G282C sample. Since the substitutions (V205M/G282C; Scheme 1, pink spheres) are away from the perturbed pathways (Scheme 1, blue crosses), existence of the unchanged slow-pathway(s) in the DV-V205M/G282C mutant is no wonder.

The delayed fluorescence intensity decreased in the DV-V205M/G282C sample (Fig. 3c; 36-ns component), indicating a decrease in the energy transfer from core antennae to PSII-RC in the DV-V205M/G282C sample. There are three bidirectional pathways from core antennae to PSII-RC; one is from CP47, and the other two from CP43 [12]. If the pathway between CP47 and PSII-RC (Scheme 1, green circle) was blocked in the DV-V205M/G282C sample, the delayed fluorescence should appear at 686 nm. However, the DV-V205M/G282C sample showed the delayed fluorescence at 688 nm. On the other hand, perturbations in the pathways from CP43 (Scheme 1, red crosses) may decrease the energy transfer to PSII-RC without disturbing the delayed fluorescence distribution to 688 nm. Therefore, we concluded that the amino acid alterations decreased the energy transfer from CP43 to PSII-RC (Scheme 1, at least one of the red crosses) in the DV-V205M/G282C sample.

Furthermore, in the DV-V205M/G282C sample, the energy transfer from Chl_{686} to Chl_{694} was accelerated (Fig. 3c; 45-ps component). If PSII exists as monomers in this sample, the energy transfer from Chl_{686} to Chl_{694} should go through PSII-RC. However, the pathways between CP43 and PSII-RC might be perturbed in the DV-V205M/G282C sample as discussed above (Scheme 1, red cross). We suspected that Chl_{686} in CP43 transfers energy to Chl_{694} in CP47, which neighbors in the PSII dimer (Scheme 1, red arrow) [21]. The distance between Chl_{694} and other chlorophylls in neighbor PSII monomer is about 31–61 Å (Fig. S6A), which is shorter than the Förster critical distance between Chl *a* molecules (80–90 Å) [22]. This newly constructed pathway also decreases energy transfer to PSII-RC by trapping energy at Chl_{694}, which may explain the partial recovery of high-light tolerance in the DV-V205M/G282C mutant [9]. The DV-V205M/G282C sample showed 10–20% faster fluorescence decay in a few ns time region at room temperature (Fig. S5), which might be related to the energy transfer to Chl_{694} and its nearby carotenoids. A previous report suggested that DV-Chl itself generates larger amounts of triplet-state DV-Chl compared with MV-Chl under the same light conditions [23]. Chl_{694} might dissipate trapped energy using nearby carotenoid pigments (Fig. S6B) [15,24]. In addition, a previous report suggested that CP47 might relate to spillover, that is, energy transfer from PSII to PSI [25]. It is likely that the DV-V205M/G282C mutant avoids accumulation of excess energy by transferring energy from CP43 to CP47 in the PSII dimer, where CP47 could quench or utilize excitation energy. These processes might become more important when the PSII-RC was in closed state. Previous report suggested that partial PSII-RC easily became closed state in vivo even under weak light condition (20 μmol of photons m^{-2} s^{-1}) [26]. We assumed that about 40% of the reaction centers were open in our experiments based on the amplitudes of shorter lifetime components (<230 ps) [27].

In this study, we analyzed the changes in energy transfer pathways caused by D1 amino acids alterations or Chl in PSII-RC of *Synechocystis* mutants. When the MV-Chls were replaced with DV-Chls, energy transfer between PSII-RC and Chl_{694} in CP47 decreased, and excitation energy accumulated around PSII-RC. The conserved amino acids in the D1 protein of *Prochlorococcus* changed the distribution of excitation energy in the PSII dimer of the *Synechocystis* DV-V205M/G282C mutant. In that mutant, the energy transfer from CP43 to PSII-RC was decreased, and CP43 may transfer excitation energy to CP47 in the PSII dimer, which may explain the partial recovery of high-light tolerance in this mutant. Since V205M and G282C were not located close to antenna Chls, these substitutions might perturb the orientation of D1 to change the energy transfer pathways.

3.3. Optimization of PSII after chlorophyll replacement

Genomic analyses revealed that the progenitor of the genus *Prochlorococcus* lost the DVR gene and acquired DV-Chl [6], which could be an advantage to survive under dim blue light condition such as the bottom of deep-sea water column [4]. Especially under oligotrophic waters that is the habitat of *Prochlorococcus* [28], accumulation of phycobilisome will become harder, then acquisition of DV-Chl might be useful to harvest light energy at low cost. Actually, all *Prochlorococcus* species lost phycobilisomes and acquired DV-Chl binding antenna complexes [29]. However, DV-Chl itself generates larger amounts of triplet-state DV-Chl compared with MV-Chl [23]. Therefore, the progenitor should have a weakness in high-light tolerance. This weakness may not become a serious problem under the initial dim blue light condition, because the DV mutant can grow well under low light condition [6,9]. Time-resolved fluorescence analyses also suggested that the replacement of MV-Chls with DV-Chls conduced to accumulation of excitation energy at the Chl_{688} instead of the Chl_{694} (Scheme 1), where the Chl_{688} was located closer to D1 compared to the Chl_{694}. This feature might be helpful to concentrate limited excitation energy to PSII-RC. On the other hand, in case the progenitor encounters high-light condition, both the larger amounts of triplet-state formation and the concentrated excitation energy to PSII-RC may become fatal, and they could cause the degradation of D1 proteins [5]. We hypothesize that the progenitor acquired partial light tolerance through decrease of the energy transfer from antennae to reaction centers, instead of re-acquirement of the MV-Chl. One contributing factor of decreased energy transfer to PSII-RC is the substitution in D1 protein (V205M/G282C). Additional factor might be evolution of high light-inducible proteins [30], which dissipate the excess energy from PSI-RC [31]. *Prochlorococcus* might give priority to prevent triplet-state formation by excess excitation energy to adapt to various light conditions in the low nutrient water. Further research is required to determine the detailed conformation of the PSII complex in *Prochlorococcus*.

Supplementary materials related to this article can be found online at doi:10.1016/j.bbabio.2012.02.009.

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