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Excitation energy transfer between photosystem II and photosystem I in red algae: Larger amounts of phycobilisome enhance spillover

Makio Yokono [a,*], Akio Murakami [b], Seiji Akimoto [a,c]

[a] Molecular Photoscience Research Center, Kobe University, Kobe 657-8501, Japan
[b] Kobe University Research Center for Inland Seas, Awaji, 656-2401, Japan
[c] JST, CREST, Kobe, 657-8501, Japan

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ABSTRACT

We examined energy transfer dynamics from the photosystem II reaction center (PSII-RC) in intact red algae cells of *Porphyridium cruentum*, *Bangia fuscopurpurea*, *Porphyra yezoensis*, *Chondrus giganteus*, and *Prionitis crispata*. Time resolved fluorescence measurements were conducted in the range of 0–80 ns at −196 °C. The delayed fluorescence spectra were then determined, where the delayed fluorescence was derived from the charge recombination between $P680^+$ and pheophytin *a* in PSII-RC. Therefore, the delayed fluorescence spectrum reflected the energy migration processes including PSII-RC. All samples examined showed prominent distribution of delayed fluorescence in PSII and PSI, which suggests that a certain amount of PSII attaches to PSI to share excitation energy in red algae. The energy transfer from PSII to PSI was found to be dominant when the amount of phycoerythrobilin was increased.

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1. Introduction

A balanced distribution of absorbed light energy between photosystem I and II (PSI, PSII) is required for maximum efficiency during oxygenic photosynthesis. State transition is a balancing mechanism that consists of two states: state 1 is induced by preferential excitation of PSI, and state 2 is induced by preferential excitation of PSII [1]. It is generally accepted that the mechanism involves a mobile light-harvesting antenna [2]. Another proposed mechanism is spillover, in which PSII transfers its excess excitation energy to PSI [3]. Green algae and cyanobacteria possess mobile light-harvesting antennae that regulate the excitation energy distribution [4,5]. Conversely, a previous report suggested that red algae may use a spillover mechanism [3].

However, it is difficult to distinguish the mobile antenna mechanism from the spillover mechanism under natural conditions. This is because the loose binding of mobile antennae and reaction centers (RC) makes it difficult to isolate and purify antenna–RC complexes. Fluorescence spectroscopy is useful for monitoring the state transition in vivo [4]. Because PSI-RC and PSII-RC have similar chromophores, excitation photons are absorbed by both reaction centers. Steady state fluorescence measurement cannot distinguish whether the mobile antenna transfers its energy to PSI directly or via PSII. Although time resolved fluorescence spectroscopy has revealed energy transfer kinetics, energy transfer from the mobile antenna to PSI and PSII is still complicated because a large number of chromophores are involved in these systems [3]. Furthermore, similar time constants (<100 ps) are observed in energy transfer from the mobile antenna to RC and energy transfer within RC [6–8]. Accordingly, it is necessary to observe excitation energy flow after direct excitation of PSI-RC or PSII-RC to examine the balancing mechanism.

PSII-RC specifically emits delayed fluorescence in the range of 15–60 ns at −196 °C [7,9–11], whereas PSI and mobile antennae show little fluorescence in this range [6,12–14]. Charge separation in the PSII-RC takes place after the excitation energy absorbed by the antenna chromophore reaches the PSII-RC. At low temperatures, direct charge recombination occurs between $P680^+$ and pheophytin *a*, and the excited state is regenerated at PSII-RC far later than the initial excitation pulse [9]. Therefore, the delayed fluorescence spectrum reflects the fluorescence spectrum generated by direct excitation of PSII-RC.

Red algae possess a large light-harvesting antenna, phycobilisome (PBS) [15], which is composed of three types of phycobiliproteins: phycoerythrin (PE), phycocyanin, (PC) and allophycocyanin (APC). PE contains two types of chromophores: phycourobilin (PUB) and phycoerythrobilin (PEB). PC and APC contain phycocyanobilin (PCB) as a chromophore, which possesses a lower excitation energy level than the two types of chromophores in PE. These chromophores absorb a wide range of light from 500 nm to 680 nm; therefore, they

Abbreviations: PSI, photosystem I; PSII, photosystem II; RC, reaction center; PBS, phycobilisome; PE, phycoerythrin; PC, phycocyanin; APC, allophycocyanin; PUB, phycourobilin; PEB, phycoerythrobilin; PCB, phycocyanobilin; Chl *a*, chlorophyll *a*; PSI red Chl, PSI red chlorophyll *a*; FDAS, fluorescence decay associated spectrum; TRFS, time resolved fluorescence spectra

* Corresponding author. Tel.: +81 80 3295 1623; fax: +81 78 803 5705.
E-mail address: filia@mac.com (M. Yokono).

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can absorb, statistically, a larger fraction of the visible light due to a very large optical cross-section in the visible region. In cyanobacteria, PBS transfers excitation energy directly to both PSII and PSI [16]. Conversely, previous studies of red algae have suggested that energy transfer might occur from PBS to PSI via PSII (spillover) [3]. However, to the best of our knowledge, there is no direct experimental evidence of energy transfer processes from PSII to PSI in vivo. In this study, we measured the time resolved fluorescence of intact cells of various red algae in the range of 0–80 ns and observed fluorescence decay associated spectra, including the delay fluorescence spectra. The delayed fluorescence spectra of various red algae showed that more than 50% of PSII transfers excitation energy to PSI. Additionally, the ratio of spillover increased in proportion to the accumulation level of PEB when compared to that of chlorophyll *a*.

2. Materials and methods

Porphyridium cruentum NIES-2138 was obtained from the National Institute for Environmental Studies, Ibaraki, Japan. Macrophytic red algae *Bangia fuscopurpurea* and *Porphyra yezoensis* were collected from the floating net of Nori-culture in Harimanada (Hyogo, Japan), and *Chondrus giganteus and Prionitis crispata* were collected on the rocky coast of Awaji Island (Hyogo, Japan). Steady state absorption spectra were recorded under a microscope (Olympus BX50) using a light guided multichannel photodiode array detector (PMA-11, Hamamatsu Photonics) at room temperature [17]. Steady state fluorescence spectra were recorded using a spectrofluorometer (FP 6600, JASCO) at −196 °C. The excitation wavelength was 400 nm to 680 nm with 5 nm intervals, and fluorescence spectra were recorded with 1 nm intervals. Time resolved fluorescence was measured using the time correlated single photon counting method at −196 °C [18]. The excitation wavelength was set at 400 nm and the repetition rate of the pulse trains was 2.9 MHz, which did not interfere with measurements taken at up to 100 ns (24.4 ps/channel × 4096 channels). To improve the time resolution, time resolved fluorescence was also measured for up to 10 ns (2.4 ps/channel × 4096 channels) using a 1 nm interval (680–700 nm) and a 2 nm interval (700–740 nm). Following global analysis of the fluorescence kinetics, fluorescence decay associated spectra (FDAS) were constructed [18].

3. Results

3.1. Steady state absorption and fluorescence spectra

Fig. 1a shows absorption spectra of *P. cruentum*, *B. fuscopurpurea*, and *P. yezoensis* normalized at 680 nm. Chlorophyll *a* showed two peaks at 440 nm and 680 nm in all samples, corresponding to the Soret and Qy bands, respectively. Three types of phycobilins, PUB, PEB, and PCB, exhibited absorption peaks around 500 nm, 560 nm, and 625 nm, respectively [18]. Carotenoids also contributed to the 500 nm peak. The intensity of the PEB peak was lower in the *P. yezoensis* sample than the *P. cruentum* and *B. fuscopurpurea* samples, which might reflect a variation of relative amount of PBS compared to that of reaction centers in individual samples.

Fig. 1b shows the fluorescence spectra of *P. cruentum* at −196 °C measured using excitation wavelengths of 400–680 nm. The 440 nm and 560 nm excitation lights were solely absorbed by chlorophyll *a* and phycoerythrobilin, respectively, whereas the 400 nm excitation light was absorbed by both phycobilins and Chl *a*[18]. The 440 nm excitation showed a peak around 715 nm, which came from red chlorophyll *a* in PSI (PSI red Chl). These findings are consistent with the previous finding that approximately 80% of the Chl *a* was attached to PSI in *P. cruentum* grown under white light [19]. Under an excitation wavelength of 400 nm, three fluorescence peaks were observed around 590 nm, 650 nm, and 715 nm. The former two were produced by PEB and PCB in PBS, respectively, while the latter one was produced by PSI red Chl. In addition, a shoulder at about 685 nm was produced by Chl *a* in PSII, which accepts excitation energy from phycobilins in addition to direct excitation by light energy itself. Under an excitation wavelength of 560 nm, only PEB in PBS absorbs the light energy, and energy transfer occurs. Four peaks were observed around 590 nm, 650 nm, 685 nm, and 715 nm, and the latter two were produced by Chl *a* in PSII and PSI red Chl, respectively. These findings indicate that excitation energy was transferred from PBS to both PSII and PSI. To determine if PBS transfers its energy to PSI directly or via PSII, we measured the time resolved fluorescence at an excitation wavelength of 400 nm.

3.2. Fluorescence decay associated spectra (FDAS)

3.2.1. FDAS of P. cruentum

Time resolved fluorescence measurements were conducted to reveal the energy transfer processes in the PSII and PSI fluorescence wavelength region. Fig. 2a shows the fluorescence decay associated spectra (FDAS) of *P. cruentum* obtained at −196 °C. The first four components, 60 ps, 100 ps, 470 ps, and 1.6 ns, reflect energy transfer kinetics including quenching of energy traps following laser excitation. In addition, the 13 ns component is a delayed fluorescence spectrum that reflects energy distribution after the charge recombination at PSII-RC [9]. In other words, the 13 ns component can be viewed as a fluorescence spectrum with direct excitation of PSII-RC.

The 60 ps component possessed a set of positive and negative peaks at 692 nm and 712 nm, indicating energy transfer to PSI red Chls. The 100 ps component showed a positive peak at 706 nm with a shoulder at 712 nm, which might reflect energy transfer around the

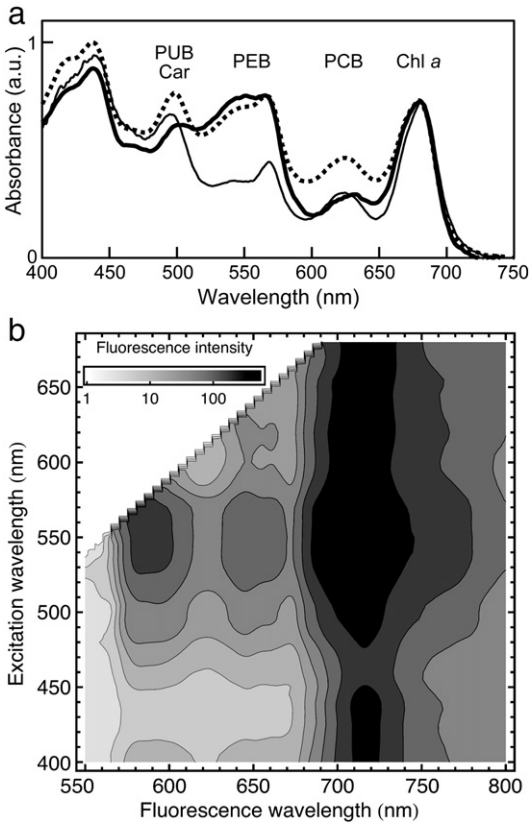


Fig. 1. a) Absorption spectra of *Porphyridium cruentum* (thick solid line), *Bangia fuscopurpurea* (thick dash line), and *Porphyra yezoensis* (thin solid line) at room temperature. Spectra were normalized at 680 nm. Car, carotenoids; PUB, phycourobilin; PEB, phycoerythrobilin; PCB, phycocyanobilin; Chl *a*, chlorophyll *a*. b) Contour map of steady state fluorescence spectra of *P. cruentum* at −196 °C. Spectra were measured at excitation wavelengths every 5 nm from 400 to 680 nm. Inset: gradation scale of fluorescence intensity.

712 nm PSI red Chl. The 712 nm red Chl also showed positive peaks in the 470 ps component. Moreover, the 1.6 ns component showed a positive peak at 722 nm, indicating a contribution of another PSI red Chl. In addition to PSI peaks, the 100 ps component showed a set of positive and negative peaks at 685 nm and 690 nm, reflecting energy transfer in PSII. However, the intensities of the PSII peaks were weak when compared to those of the PSI peaks.

The 13 ns component is a delayed fluorescence spectrum that reflects the excitation energy distribution after charge recombination in PSII-RC. Three peaks were observed at 686 nm, 696 nm, and 712 nm. The former two peaks were consistent with those of the PSII cores isolated from spinach and *Synechocystis* sp. PCC 6803 [9]. Surprisingly, the delayed fluorescence that originated in charge recombination in PSII showed a large peak at 712 nm from PSI red Chl. These findings clearly demonstrate that energy transfer from PSII to PSI, i.e., spillover, occurred. The ratio of the integrated intensity of the PSI peak to the PSII peaks was 2.9 (summarized in Table 1), which may reflect the proportion of PSII–PSI complex.

3.2.2. FDAS of *B. fuscopurpurea*

The results observed in other species of red algae were nearly identical to those observed in *P. cruentum*. Fig. 2b shows the FDAS of *B. fuscopurpurea* at −196 °C. The 50 ps component possessed a broad positive peak around 700 nm and negative peaks at 715 nm and 725 nm, indicating energy transfer to PSI red Chls. The 360 ps component showed two sets of positive and negative peaks. The former peaks were at 685 nm and 695 nm, which were similar to the results observed for *P. cruentum* and reflected energy transfer in PSII. The latter peaks were at 715 nm and 735 nm, indicating slow energy transfer between PSI red Chls. In the 1.2 ns and 2.8 ns components, PSI red Chls showed positive peaks around 730 nm.

The 29 ns component is a delayed fluorescence spectrum that reflects excited energy distribution following the charge recombination in PSII-RC. Three peaks were observed at 685 nm, 695 nm, and 725 nm. The delayed fluorescence showed a large peak in the PSI red Chl region, as is the case for *P. cruentum*. The ratio of the integrated intensity of the PSI peak to the PSII peaks was 2.4.

3.2.3. FDAS of *P. yezoensis*

Fig. 2c shows the FDAS of *P. yezoensis* at −196 °C. The observed kinetics were similar to those of *B. fuscopurpurea*, with the following exceptions: a) PSII peaks appeared to be more clear, and the ratio of integrated intensity of the PSI peak to the PSII peaks was 1.9 in the 20 ns components; b) the deepest red Chl in PSI showed a fluorescence maxima at 738 nm. The latter finding reflects the diversity of the red Chls in various photosynthetic organisms of red algae. The former result reflects efficient light-harvesting in PSII, and/or suppressed spillover. Here, *P. yezoensis* showed the lower accumulation level of PEB (Fig. 1a, thin solid line), which suggests the limited light-harvesting ability of PSII. Therefore, spillover was reduced in *P. yezoensis* sample.

3.2.4. FDAS of *C. giganteus* and *P. crispata*

To elucidate the correlation between the accumulation level of PEB and spillover while excluding the differences between species, we selected *C. giganteus* and *P. crispata* as additional samples. Fig. 3a and b shows photographs of these samples. Both species have flabellate thalli with a cuneate base. There were color variations in these samples, with the *C. giganteus* sample appearing purple around the base and gradually becoming green going toward the tip, while the *P. crispata* sample was green at the base and purple at the tip. These features were useful for comparison of the accumulation level of phycoerythrobilin and the degree of spillover in the same species. The absorption spectra of the purple colored part showed a clearly higher absorption intensity at around 550 nm when compared to the green colored part (Fig. 3c and d). These findings reflected the higher amount of PEB in the purple colored part of the organism.

Fig. 4 shows the FDAS of *C. giganteus* and *P. crispata* at −196 °C. All samples showed similar kinetics in the four components, 50–60 ps, 110–180 ps, 610–890 ps, and 2.2–2.7 ns. A difference was observed in the components with the longest lifetime, which were the delayed fluorescence spectra. In *C. giganteus* samples, the delayed fluorescence spectra (12 ns for purple and 18 ns for green) showed three peaks at 686 nm, 696 nm, and 716 nm, which were assigned to the PS II, PS II, and PS I fluorescences, respectively. For this organism, the PSI peak was prominent in the purple colored part. The ratio of the integrated intensity of the PSI peak to the PSII peaks was 4.0 for the purple colored part, while it was 1.2 for the green colored part. The *P. crispata* samples also showed similar patterns to the delayed fluorescence spectra (14 ns for purple, and 13 ns for green), with three peaks being observed at 686 nm, 696 nm and 718 nm. In addition, the relative intensity of the PSI peak compared to the PSII peaks was higher in the

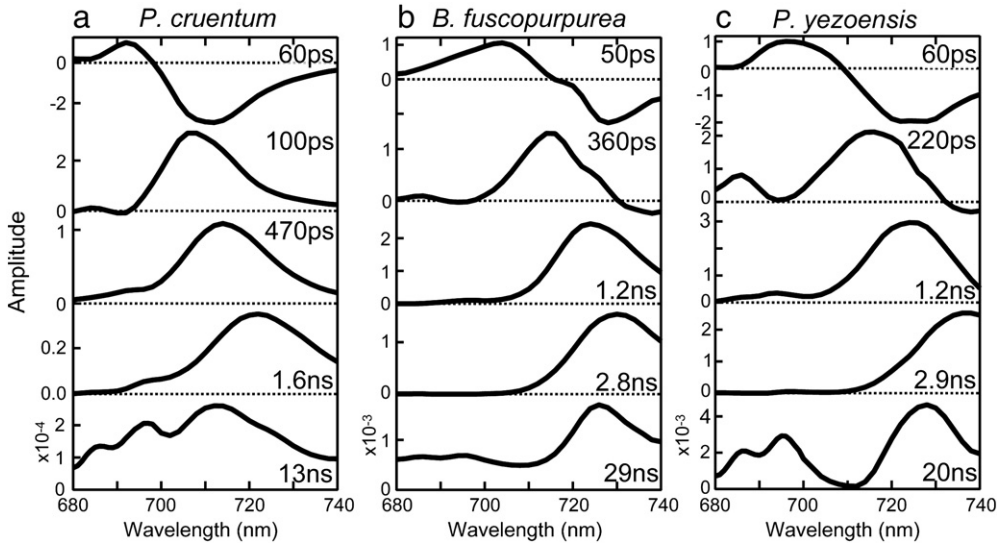


Fig. 2. FDAS of *Porphyridium cruentum*, *Bangia fuscopurpurea*, and *Porphyra yezoensis* at 77 K. The amplitudes were normalized at short lived component. Positive and negative peaks correspond to fluorescence decay and rise components, respectively.

Table 1
Excitation energy distribution and antenna size.

	P. cruentum	*B. fuscopurpurea*	*P. yezoensis*	*C. giganteus* (purple part)	*C. giganteus* (green part)	*P. crispata* (purple part)	*P. crispata* (green part)
Delayed fluorescence intensity ratio (PSI peak/PSII peak)	2.9	2.4	1.9	4.0	1.2	2.5	1.1
Fluorescence mean lifetime ratio (PSI peak/PSII peak)	1.0	1.2	1.7	2.7	1.5	1.5	1.3
Estimated ratio of PSII–PSI complex/independent PSII	2.9	2.0	1.1	1.5	0.80	1.7	0.85
Absorbance ratio (PEB/Chl *a*)	0.75	0.7	0.35	0.31	0.09	0.34	0.13

purple colored part for *P. crispata*, and the ratio of the integrated intensity of the PSI peak to the PSII peaks was 2.5 for purple and 1.1 for green.

4. Discussion

The steady state fluorescence spectra with excitation around 500–600 nm (PBS excitation) showed PBS–PSII peaks (590–685 nm), as well as a PSI red Chl peak (~715 nm) (Fig. 1b). These findings indicate that excitation energy was transferred from PBS, and that both PSII and PSI worked as final energy traps. Two energy transfer pathways are possible from PBS to PSI: direct transfer from PBS to PSI (PBS → PSI) and sequential transfer via PSII (PBS → PSII → PSI).

We can recognize energy transfer from PSII to PSI by measuring the delayed fluorescence spectrum at −196 °C. The delayed fluorescence showed lifetimes longer than 10 ns, which originated from an excited state that was populated by charge recombination at the level of the photochemical trap of PSII [9,11]. Additionally, delayed fluorescence was not detected in isolated PS I because charge recombination does not occur due to rapid electron transfer from the primary electron acceptor to the secondary electron acceptor [14]. The 12–29 ns components of FDAS are the delayed fluorescence spectra of red algae intact cells (Figs. 2 and 4). All delayed fluorescence spectra showed a PSI peak around 710–730 nm in addition to PSII peaks at 686 nm and 696 nm. The PSI peak in the delayed fluorescence spectra indicates energy transfer from PSII to PSI following the charge recombination in PSII-RC. Moreover, the existence of PSII peaks indicates that there is a PSII system that did not transfer its excitation energy to PSI. As discussed later, only the 50–60 ps component contains signals that can be assigned due to the energy transfer from PSII to PSI, and contribution of slow spillover is not recognized. The time constant of spillover, 50–60 ps, is ten times shorter than the mean lifetime of isolated PSII complex at −196 °C [9]. Therefore, it is expected that the ratio of intensities of PSII and PSI peaks in the delayed fluorescence spectrum mainly reflects a ratio of spillover.

The time constant of spillover is not necessarily clear at the present experimental stage; however, the 50–60 ps component is the most probable candidate. This is because the 50–60 ps components of FDAS showed a set of positive and negative peaks around 690 nm and 710–730 nm in all samples (Figs. 2 and 4), which reflects efficient energy transfer to the PSI red Chl. If excitation energy transfer occurred from PSII to PSI (spillover), the intensities of PSII fluorescence would decrease and those of PSI fluorescence would increase independently of the existence of a PBS–PSI direct transfer. In FDAS of *B. fuscopurpurea* and *P. yezoensis* samples (Fig. 2b and c), the 360 ps and the 220 ps components also showed a negative peak at 735 nm

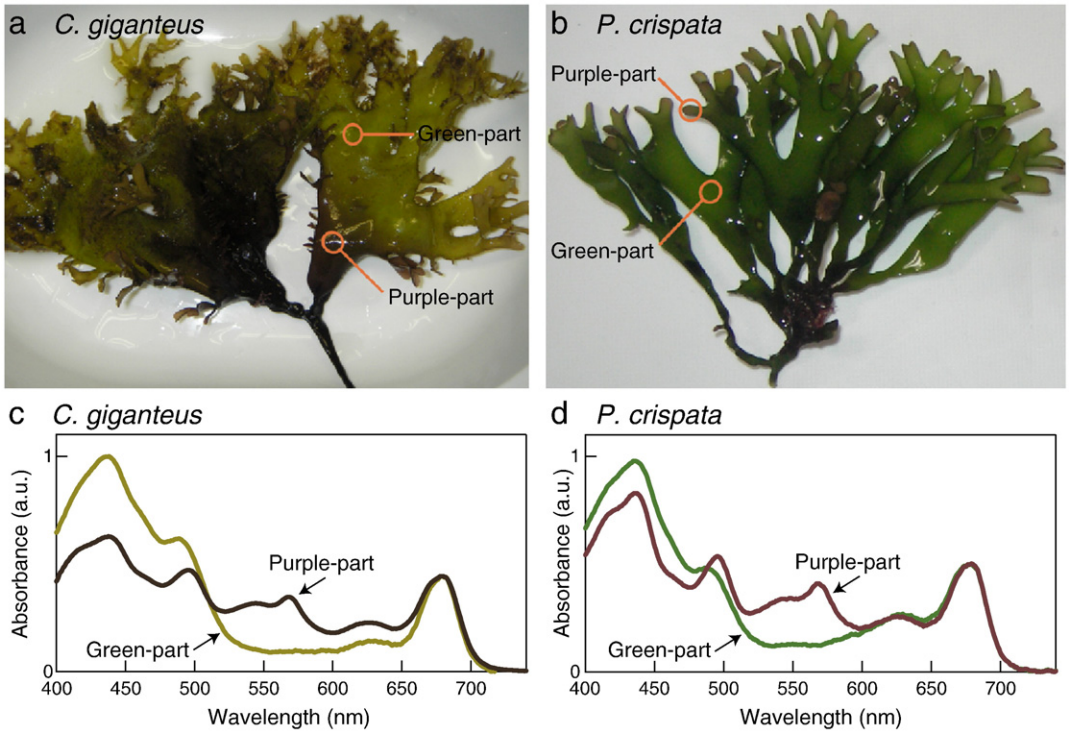


Fig. 3. Photographs and absorption spectra of color variants of *Chondrus giganteus* (a) and *Prionitis crispata* (b) thalli. Both species have purple and green colored portions in the same thalli.

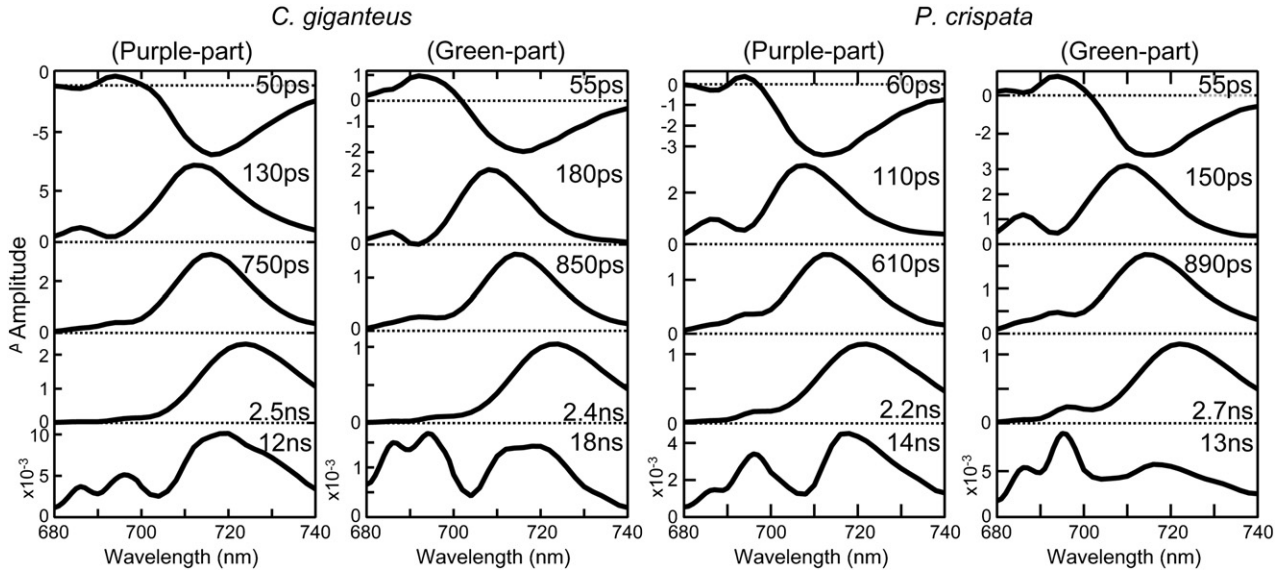


Fig. 4. FDAS of *Chondrus giganteus* and *Prionitis crispata* at 77 K. Purple and green colored parts were measured individually (see Fig. 3). The amplitudes were normalized against the short lived components. Positive and negative peaks correspond to fluorescence decay and rise components, respectively.

and 738 nm, which reflects slow energy transfer to the deepest red Chl of PSI. Here, the delayed fluorescence spectra did not show a positive peak of the deepest red Chl. Therefore, the slow energy transfer might not act as a main pathway of spillover.

The possible time constant of spillover, 50–60 ps, was observed at −196 °C. At low temperature, only downhill energy transfer, from PSII to PSI red Chl, should be observed. On the other hand, at room temperature, both downhill and uphill pathways become effective [20]. Therefore, Chl in PSII could share excitation energy captured by Chl in PSI through PSI-to-PSII uphill energy transfer at room temperature when the amount of PBS becomes too small under several environmental conditions [21].

In the delayed fluorescence spectra, the ratio of the integrated intensity of the PSI peak to the PSII peaks was higher in the purple colored parts than the green colored parts (Fig. 4). This trend suggests that there was enhanced spillover in response to the larger accumulation of PBS. PBS contains PUB, PEB, and PCB, which have absorption maxima of approximately 500 nm, 550 nm, and 630 nm, respectively. Here, Car showed an absorption maximum of 500 nm, and Chl *a* exhibited a Q_X band around 630 nm. Therefore, we selected the relative absorbance at 550 nm as an indicator of the amount of PBS (Figs. 1 and 3, and Table 1). It was previously reported that *P. cruentum* does not change the phycobiliproteins' composition under various light condition [19]. In this case, the amount of PBS reflects the number of PBS. However, as shown in Fig. 3d, the purple colored part of *P. crispata* showed larger PE peak around 550 nm without clear change in PC peak around 630 nm compared to the green colored part, indicating difference of the phycobiliproteins composition between each parts. The amount of PBS may not be equal to the number of PBS, but it mostly reflects the peripheral PSII antenna size. The inset in Fig. 5 shows the relationship between the relative amount of PBS and the ratio of the delayed fluorescence intensity (PSI red Chl wavelength region/PSII wavelength region). We observed a weak correlation with a correlation coefficient of 0.49. Here, the ratio of delayed fluorescence intensity reflected the amount of PSII that interacted with PSI; however, the ratio was also affected by the fluorescence quantum yield of both PSII Chls and PSI red Chls. The fluorescence quantum yield of monomeric Chl *a* varies with environmental conditions including solvents or proteins, showing a lifetime range of 2–7 ns as the singlet excited state lifetime [22–26]. We assumed that the fluorescence quantum yield was roughly proportional to the fluorescence mean lifetime in the range of <7 ns. The delayed fluorescence lifetime mainly reflected electron transfer steps, and the threshold of <7 ns only omitted the delayed fluorescence component from calculation of the mean lifetime in our data. Table 1 summarizes the ratio of the fluorescence mean lifetimes of PSI red Chls to that of PSII Chls. In the purple part of *C. giganteus*, the ratio of the integrated intensity of the PSI peak to the PSII peaks was 4.0, but the mean lifetime was also 2.7 times longer in the PSI red Chl region. These findings suggest that the excitation energy in PSI red Chl has a 2.7 times greater probability of fluorescence emission than that in PSII. Therefore, a corrected value of 4.0/2.7 = 1.5 more accurately represents the ratio of PSII that interacts with PSI in the purple part of *C. giganteus* sample. The ratios in the other samples were also corrected (Table 1). Fig. 5 shows the relationship between the estimated ratio of PSII that interacts with PSI and the amount of PBS, which showed a correlation coefficient of 0.92. The ratio of the PSII–PSI complex to the independent PSII complex ranged from 0.8 to 2.9,

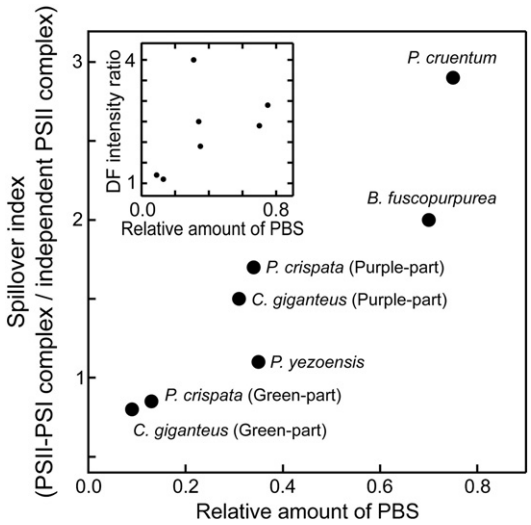
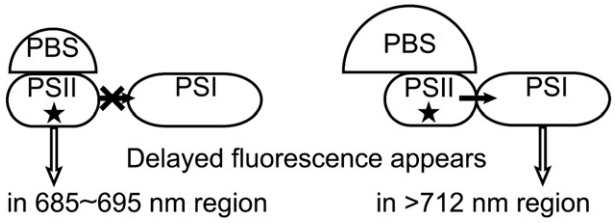


Fig. 5. Estimated relationship between relative amount of phycobilisome and spillover. The relative amount of phycobilisome was represented by the absorbance ratio (A_{550}/A_{680}). The spillover index was estimated from the delayed fluorescence intensity and mean lifetime in the PSI and PSII wavelength region. Inset: relationship between the relative amount of phycobilisome and delayed fluorescence intensity ratio (PSI wavelength region/PSII wavelength region).



Scheme 1. Estimated relationship between PSII antenna size and spillover. Stars represent excited state generated by charge recombination in PSII. Black arrows represent excitation energy transfer processes after charge recombination in PSII. Delayed fluorescence appears following charge recombination and the energy transfer process (white arrows).

which indicates that more than 45% of the PSII shared the excitation energy with PSI in our samples. The various species harvested from their own fields showed a single correlation between the relative amount of PBS and the ratio of the PSII–PSI complex. A larger amount of PBS increased the contribution of spillover (Scheme 1). Red algae may use PBS as an antenna for both PSII and PSI via the spillover mechanism [3]. A larger amount of PEB indicates a higher light-harvesting probability in PBS, which causes an increase in PS II antenna size; therefore, red algae use the spillover mechanism to transfer energy to PSI. In addition, a previous report suggested that *P. cruentum* changes the relative numbers of PSI and PSII per PBS depending on growth light conditions [19]. This acclimation is effective; however, it requires time to synthesize RC [27]. The delayed fluorescence spectra in the present study revealed that various species of red algae used the spillover mechanism. When PSII was attached to PSI, Fv/Fm could decrease due to additional competitive traps including P700. It has been suggested that *P. cruentum* shows a rapid reversible change in Fv/Fm in response to light wavelength [27,28]. The degradation curve of PSII shows slower kinetics when compared to the change of Fv/Fm during the early stages [27]; therefore, another mechanism may account for the rapid acclimation. Accordingly, we can conclude that spillover plays a role in the rapid acclimation to light in red algae.

The delayed fluorescence lifetime varied from 12 to 29 ns in our samples. This is not surprising because isolated PSII particles from various photosynthetic organisms have also shown individual delayed fluorescence lifetime in the range of 15–60 ns [9–11,29–31]. One possibility of lifetime variation is the difference in the energy migration pathway following charge recombination in PSII-RC [32]. This variation could also be influenced by the open/closed state of PSII-RC [10].

In this study, we demonstrated that red algae utilized spillover to share the excitation energy between two photosystems, PSII and PSI. The ratio of PSII–PSI complex to the PSII complex ranged from 0.8 to 2.9, and this ratio was related to the light-harvesting ability of PBS. Further studies are necessary to determine the detailed conformation of the PSII–PSI complex.

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References

[1] F. Wollman, State transitions reveal the dynamics and flexibility of the photosynthetic apparatus, EMBO J. 20 (2001) 3623–3630.
[2] C.W. Mullineaux, Phycobilisome-reaction centre interaction in cyanobacteria, Photosynth. Res. 95 (2008) 175–182.
[3] J. Biggins, D. Bruce, Regulation of excitation energy transfer in organisms containing phycobilins, Photosynth. Res. 20 (1989) 1–34.
[4] M. Iwai, M. Yokono, N. Inada, J. Minagawa, Live-cell imaging of photosystem II antenna dissociation during state transitions, Proc. Natl. Acad. Sci. USA 107 (2010) 2337–2342.
[5] R. Zhang, H. Li, J. Xie, J. Zhao, Estimation of relative contribution of "mobile phycobilisome" and "energy spillover" in the light–dark induced state transition in *Spirulina platensis*, Photosynth. Res. 94 (2007) 315–320.
[6] G. Hastings, F.A.M. Kleinherenbrink, S. Lin, R.E. Blankenship, Time-resolved fluorescence and absorption spectroscopy of photosystem I, Biochemistry 33 (1994) 3185–3192.
[7] L. Konermann, G. Gatzen, A.R. Holzwarth, Primary processes and structure of the photosystem ii reaction center. Part V. Modeling of the fluorescence kinetics of the D1–D2–cyt-b559 complex at 77 K, J. Phys. Chem. B 101 (1997) 2933–2944.
[8] C.W. Mullineaux, E. Bittersmann, J.F. Allen, A.R. Holzwarth, Picosecond time-resolved fluorescence emission spectra indicate decreased energy transfer from the phycobilisome to photosystem II in light-state 2 in the cyanobacterium *Synechococcus* 6301, Biochim. Biophys. Acta Bioenerg. 1015 (1990) 231–242.
[9] M. Mimuro, S. Akimoto, T. Tomo, M. Yokono, H. Miyashita, T. Tsuchiya, Delayed fluorescence observed in the nanosecond time region at 77 K originates directly from the photosystem II reaction center, Biochim. Biophys. Acta Bioenerg. 1767 (2007) 327–334.
[10] E. Romero, I. van Stokkum, V. Novoderezhkin, J. Dekker, R. Van Grondelle, Two different charge separation pathways in photosystem II, Biochemistry 49 (2010) 4300–4307.
[11] P. Booth, B. Crystall, L. Giorgi, J. Barber, D. Klug, G. Porter, Thermodynamic properties of D1/D2/cytochrome b-559 reaction centres investigated by time-resolved fluorescence measurements, Biochim. Biophys. Acta Bioenerg. 1016 (1990) 141–152.
[12] E. Bittersmann, W. Vermaas, Fluorescence lifetime studies of cyanobacterial photosystem II mutants, Biochim. Biophys. Acta Bioenerg. 1098 (1991) 105–116.
[13] B. van Oort, A. van Hoek, A.V. Ruban, H. van Amerongen, Aggregation of light-harvesting complex II leads to formation of efficient excitation energy traps in monomeric and trimeric complexes, FEBS Lett. 581 (2007) 3528–3532.
[14] M. Mimuro, M. Yokono, S. Akimoto, Variations in photosystem I properties in the primordial cyanobacterium *Gloeobacter violaceus* PCC 7421, Photochem. Photobiol. 86 (2010) 62–69.
[15] A.A. Arteni, L.N. Liu, T.J. Aartsma, Y.Z. Zhang, B.C. Zhou, E.J. Boekema, Structure and organization of phycobilisomes on membranes of the red alga *Porphyridium cruentum*, Photosynth. Res. 95 (2008) 169–174.
[16] C. Dong, A. Tang, J. Zhao, C.W. Mullineaux, G. Shen, D.A. Bryant, ApcD is necessary for efficient energy transfer from phycobilisomes to photosystem I and helps to prevent photoinhibition in the cyanobacterium *Synechococcus* sp. PCC 7002, Biochim. Biophys. Acta Bioenerg. 1787 (2009) 1122–1128.
[17] S. Akimoto, A. Murakami, M. Yokono, K. Koyama, T. Tsuchiya, H. Miyashita, I. Yamazaki, M. Mimuro, Fluorescence properties of the chlorophyll d-dominated cyanobacterium *Acaryochloris* sp. strain Awaji, J. Photochem. Photobiol., A: Chemistry 178 (2006) 122–129.
[18] M. Yokono, S. Akimoto, K. Koyama, T. Tsuchiya, M. Mimuro, Energy transfer processes in *Gloeobacter violaceus* PCC 7421 that possesses phycobilisomes with a unique morphology, Biochim. Biophys. Acta Bioenerg. 1777 (2008) 55–65.
[19] F. Cunningham Jr., R. Dennenberg, P. Jursinic, E. Gantt, Growth under red light enhances photosystem II relative to photosystem I and phycobilisomes in the red alga *Porphyridium cruentum*, Plant Physiol. 93 (1990) 888–895.
[20] A.N. Melkozernov, J. Kargul, S. Lin, J. Barber, R.E. Blankenship, Spectral and kinetic analysis of the energy coupling in the PS I-LHC I supercomplex from the green alga *Chlamydomonas reinhardtii* at 77 K, Photosynth. Res. 86 (2005) 203–216.
[21] A.R. Grossman, M.R. Schaefer, G.G. Chiang, J.L. Collier, The phycobilisome, a light-harvesting complex responsive to environmental conditions, Microbiol. Mol. Biol. Rev. 57 (1993) 725–749.
[22] J. Ide, D. Klug, W. Khlbrandt, L. Giorgi, G. Porter, The state of detergent solubilised light-harvesting chlorophyll-a/b protein complex as monitored by picosecond time-resolved fluorescence and circular dichroism, Biochim. Biophys. Acta Bioenerg. 893 (1987) 349–364.
[23] F.J. Kleima, C.C. Gradinaru, F. Calkoen, I.H.M. van Stokkum, R. van Grondelle, H. van Amerongen, Energy transfer in LHCII monomers at 77 K studied by sub-picosecond transient absorption spectroscopy, Biochemistry 36 (1997) 15262–15268.
[24] M. Komura, Y. Shibata, S. Itoh, A new fluorescence band F689 in photosystem II revealed by picosecond analysis at 4–77 K: function of two terminal energy sinks F689 and F695 in PS II, Biochim. Biophys. Acta Bioenerg. 1757 (2006) 1657–1668.
[25] L.-O. Pålsson, S.E. Tjus, B. Andersson, T. Gillbro, Energy transfer in photosystem I. Time resolved fluorescence of the native photosystem I complex and its core complex, Chem. Phys. 194 (1995) 291–302.
[26] C.D. van der Weij-de Wit, J.A. Ihalainen, R. van Grondelle, J.P. Dekker, Excitation energy transfer in native and unstacked thylakoid membranes studied by low temperature and ultrafast fluorescence spectroscopy, Photosynth. Res. 93 (2007) 173–182.
[27] Y. Fujita, Chromatic variation of the abundance of PSII complexes observed with the red alga *Prophyridium cruentum*, Plant Cell Physiol. 42 (2001) 1239–1244.
[28] Y. Fujita, An evidence indicating that a weak orange light absorbed by phycobilisomes causes inactivation of PSII in cells of the red alga *Porphyridium cruentum* grown under a weak red light preferentially exciting Chl a, Plant Cell Physiol. 40 (1999) 924–932.

[29] T. Tomo, S. Akimoto, H. Ito, T. Tsuchiya, M. Fukuya, A. Tanaka, M. Mimuro, Replacement of chlorophyll with di-vinyl chlorophyll in the antenna and reaction center complexes of the cyanobacterium *Synechocystis* sp. PCC 6803: characterization of spectral and photochemical properties, Biochim. Biophys. Acta Bioenerg. 1787 (2009) 191–200.
[30] M. Szczepaniak, J. Sander, M. Nowaczyk, M.G. M̧ller, M. R˜gner, A.R. Holzwarth, Charge separation, stabilization, and protein relaxation in photosystem II core particles with closed reaction center, Biophys. J. 96 (2009) 621–631.
[31] M. Mimuro, S. Akimoto, T. Gotoh, M. Yokono, M. Akiyama, T. Tsuchiya, H. Miyashita, M. Kobayashi, I. Yamazaki, Identification of the primary electron donor in PS II of the Chl *d*-dominated cyanobacterium *Acaryochloris marina*, FEBS Lett. 556 (2004) 95–98.
[32] M. Brecht, V. Radics, J. Nieder, R. Bittl, Protein dynamics-induced variation of excitation energy transfer pathways, Proc. Natl. Acad. Sci. USA 106 (2009) 11857–11861.