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

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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.

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

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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

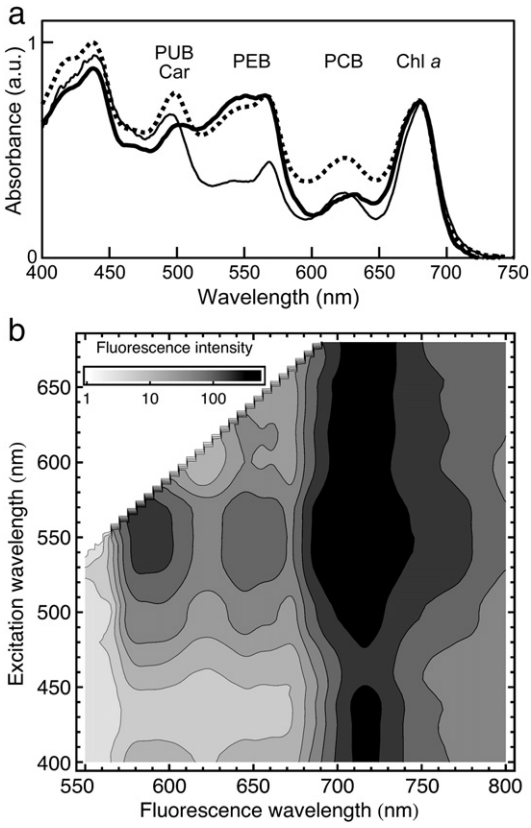

Fig. 1. a) Absorption spectra of *Porphyridium cruentum* (thick solid line), *Bangia fuscopurpurea* (thick dash line), and *Porphyra yezoensis* (thin solid line) at room temperature. Spectra were normalized at 680 nm. Car, carotenoids; PUB, phycourobilin; PEB, phycoerythrobilin; PCB, phycocyanobilin; 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.