



Light-harvesting and energy-transfer processes in chlorophyll b-containing cyanobacteria grown under the different light conditions

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Doctoral Dissertation

Light-harvesting and energy-transfer processes in
chlorophyll *b*-containing cyanobacteria grown under the
different light conditions

(クロロフィル *b* を持つシアノバクテリアの異なる光環境下における光捕集
およびエネルギー移動過程)

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1. Introduction

1.1. Photosystems and peripheral antenna systems

Oxygenic photosynthetic organisms possess two photosystems in the thylakoid membrane and the peripheral antenna in or on the thylakoid membrane.¹ Light energy is captured by the antenna pigments in the peripheral antenna systems and the two photosystems, and the energy is transferred to the reaction centers of the photosystems. The excitation energy captured by the pigments in higher energy level is transfer to those in lower energy level, at the same time moving towards the reaction center. The vast majority of the pigments in the photosynthetic organisms work as the antenna pigments. The antenna systems increase the amount of energy captured compared to a single pigment, because the various pigments and pigment-protein complexes have intrinsic absorption wavelength ranges. After the excitation of the reaction center pigments, charge separation reaction occurs and the electron transfer is invoked. Electrons are removed from water by Photosystem II (PSII), oxidizing it to molecular oxygen. The electrons extracted from water are transported to Photosystem I (PSI), and reduce the electron acceptor NADP^+ to NADPH. Protons are also transported

across thylakoid membrane to the thylakoid lumen, creating a proton gradient for the synthesis of ATP.

Photosynthetic pigments have important roles in photosynthetic organisms. Various chlorophylls (Chls) in photosynthetic organisms share photosynthetic roles in light harvesting, excitation energy transfer, and electron transfer. The molecular structures of Chls are shown in Figure 1-1. The types of Chl may depend on the light conditions of the habitat of the photosynthetic organisms. Chl *a* is the typical Chl possessed by all oxygenic photosynthetic organisms, whereas other Chls are found in some specific organisms. For example, green algae and land plants possess Chl *b*, diatoms, brown algae, and dinoflagellates possess Chl *c*, and Chl *d* and/or Chl *f* are possessed by some cyanobacteria.^{1,2} Because the transition energies of Chl *b* and Chl *c* are higher than that of Chl *a*, both pigments transfers excitation energy to Chl *a* with high efficiency.³⁻¹¹ Although Chl *d* and Chl *f* show lower Qy energy levels than Chl *a*, they can share excitation energy with Chl *a* to utilize light energy in the far-red region.¹²⁻¹⁵ Other than Chls, several types of carotenoids (Cars) are contained in the light-harvesting complexes or reaction centers.¹⁶ They are reported to have a function of

light harvesting as well as that of protection from the excess light energy.¹⁷⁻¹⁹

Fucoxanthin (Fx) is found in brown algae and diatoms, and peridinine (Peri) is in dinoflagellates. Many cyanobacteria contain β -carotene and zeaxanthin as the major Car, and green algae or land plants contain β -carotene, lutein, neoxanthin, violaxanthin, and siphonaxanthin as the major Cars. The molecular structures of the Cars are shown in Fig. 1-2.

Several types of peripheral antenna proteins possessing photosynthetic pigments are bound to the photosystems in oxygenic photosynthetic organisms, in order to broaden the utilizable wavelength range. Most cyanobacteria possess phycobilisome (PBS) composed of phycobiliproteins.²⁰ The molecular structure of the chromophores attached to the phycobiliproteins are phycocyanobilin, phycoerythrobilin, phycoviliobilin, and phycourobilin.²¹ The molecular structure of them are shown in Fig. 1-3. PBS is attached on thylakoid membrane, basically binding to PSII. However, under the condition of iron deficiency, the PBS is degraded and the alternative antenna protein IsiA or CP43' is induced. In some cyanobacteria, the supercomplex of 18-mer IsiA-PSI is reported.²²⁻²⁵ Green algae and land plants possess light-harvesting Chl protein

complex (LHC)I and LHCII as the peripheral antenna of PSI and PSII, respectively. LHCI and LHCII contains Chl *b* as well as Chl *a*.²⁷⁻²⁹ Diatom and brown algae possess fucoxanthin-Chl *a/c*-protein (FCP), though the information for the association of FCP with photosystems is limited.^{10,26,30,31} Dinoflagellates possess water-soluble peridinin-Chl *a*-protein (sPCP), which is loosely attached on the luminal side of thylakoid membrane, and intrinsic peridinin-Chl *a/c*-protein (iPCP), which is firmly embedded in the thylakoid membrane.³² The structures of some of the peripheral antenna or the supercomplexes with photosystems are shown in Fig. 1-4.

1.2 Photosynthetic organisms under the specific light conditions

The characteristics of photosynthetic organisms can be affected by the conditions of their habitat, especially the light conditions at the habitat. The effects of the light color on the growth, production of lipid or fatty acid, and light-harvesting functions have been investigated for several photosynthetic organisms. The growth of the cyanobacterium *Fremyella diplosiphon* under green or red light resulted in change of pigment compositions.^{33,34} The cells grown under green light accumulated

phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC) in PBS, whereas those grown under red light only accumulated PC and APC even though the energy-transfer processes observed in PC and APC were identical for the two light conditions; this phenomenon is known as complementary chromatic adaptation.^{33,34} The cyanobacterium *Arthrospira platensis* (*Spirulina platensis*) grown under a fluorescent lamp and light-emitting diodes (LEDs) of different colors resulted in modification of the ratios of the pigment content, PBS/Chl (and Car/Chl).^{35,36} Under the light that PBS does not absorb (blue and far-red lights), the relative amount of PBS was larger. In addition, for *A. platensis*, the modification of the relative amounts of Chl/PBS under different colored LEDs was investigated. Under green or blue LEDs, the amount of Chl clearly decreased compared with that under red, white, and yellow LEDs; the change in the amount of PBS was unclear.³⁷ Cyanobacterium *Synechocystis* sp. PCC6803 adapted in short time to PSI-selective red light weakened the interaction between the photosystems, because of the direct excitation of PSI.³⁸ The green alga *Chlamydomonas reinhardtii* grown under filtered light revealed that the light color affected the amount of total Chl per cell as well as the relative amount between PSII and PSI (monitored by the relative

amount of primary electrons accepting plastoquinone of PSII and PSI reaction center Chl).³⁹ PSII-selective light ($475 < \lambda < 700$ nm with a maximum at 580 nm) induced more total Chl amount and a higher PSI/PSII ratio than PSI-selective light ($\lambda > 650$ nm). The modification of the content ratio was thought to balance the uneven distribution of excitation. In the green alga *Dunaliella salina* grown under red and blue LEDs of various intensities, the balance of Cars changed depending on the light intensity.⁴⁰ Under red light, the amount of lutein and β -carotene was at maximum at an intensity of $128 \mu\text{mol photons m}^{-2}\text{s}^{-1}$, and the same tendency was observed for Chl *b*. Red alga *Cyanidioschyzon merolae* grown under the different color LED showed different relative intensity of PSI delayed fluorescence.⁴¹ Finally, the effects of LED color on the growth rate or lipid and fatty acid contents were reviewed for the potential application of photosynthetic organisms in photobioreactors.⁴²

1.3 Chl *a/b*-containing cyanobacteria inhabiting under specific light conditions

Prochloron, *Prochlorothrix*, and *Prochlorococcus* are unique cyanobacteria that contain both Chl *a* and Chl *b* (*Prochlorococcus* contains 3,8-divinyl (DV) Chls *a* and *b*), while in most cyanobacteria, Chl *a* and phycobilins are the major photosynthetic pigments.^{43–45} They contain a unique photosystem antenna Pcb (Prochlorophyte chlorophyll-binding protein), but do not have PBSs, although it was found that *Prochlorococcus* CCMP 1375 have trace amount of PE-like component.⁴⁶ They were previously classified as Prochlorophyta on account of their unique pigment compositions, but molecular phylogenetics now places them among the cyanobacteria.^{47–50} Green algae and land plants also have both Chl *a* and Chl *b*, but their light-harvesting systems are LHCI and LHCII.^{27–29} The light-harvesting mechanisms in green algae and land plants have been elucidated by ultrafast time-resolved fluorescence measurements, and Chl *b* is known to transfer excitation energy to Chl *a* with high efficiency (almost unity).^{5,6,9} Cyanobacterial Pcb and eukaryotic LHC differ both evolutionally and structurally.^{27–29,51} Pcb antenna is encoded by *pcb* gene, while the other Chl *a/b* binding antenna, LHC, is encoded by *lhc* (*cab*) gene. In the structural and

phylogenetic characteristics, Pcb is close to IsiA or CP43'.⁵¹ Pcb-PSI and Pcb-PSII supercomplexes in *Prochloron* are observed, but the types of Pcb in *Prochloron* is not identified yet.⁵² *Prochlorothrix* contains three types of Pcb, PcbA, PcbB, and PcbC, encoded by genes of *pcbA*, *pcbB*, and *pcbC*.⁵³ *Prochlorococcus* is reported to have eight pcb genes (*pcbA* through *pcbH*), and there is the possibility to adjust the number or types of Pcb bound to the photosystems, depending on the light condition.⁵⁴

Prochlorothrix is a free-living cyanobacterium, whereas *Prochloron* is anomalous in that it lives as an obligate symbiont in colonial ascidians inhabiting coral reefs in tropical/subtropical areas; free-living *Prochloron* has not been reported to date.^{43,44} Moreover, *Prochloron* have not been able to be reliably cultured *in vitro*. The classification of *Prochloron* remains controversial. In molecular phylogeny based on 16S rRNA gene sequencing, no significant differences have been found between *Prochloron* cells from different host species and from different sites.⁵⁵ In contrast, three morphological groups of *Prochloron* have been described in various host ascidians⁵⁶: The distribution of *Prochloron* cells in the host ascidians are described in Fig. 1-5. Group I are the *Prochloron* cells with small 'vacuoles' filling the central region of the

cell; Group II are those containing a large central 'vacuole', and Group III are those with little 'vacuolation' and many granular inclusions. Group I *Prochloron* inhabit the colony surface or the tunic of the host ascidian. Tunic is an integumentary extracellular matrix in which the ascidian zooids are entirely embedded. On the other hand, Group-II is always distributed among peribranchial–cloacal cavities of the host colony. Group-III is exclusively seen in *Didemnum molle*. These different subcellular microenvironments may affect the different photosynthetic properties between the cells of *Prochloron*.

Prochlorococcus marinus (*Prochlorococcus*) is an even more unique free-living picocyanobacterium that contains divinyl chlorophylls (DV-Chls), DV-Chl *a* and DV-Chl *b*, as the main photosynthetic pigments.^{57–62} The absorbance maximum in the Soret band of DV-Chls is red-shifted by approximately 10 nm from that of monovinyl Chls (MV-Chls), whereas the Qy bands do not show clear shifts.⁶³ In the previous work, the light-harvesting mechanisms of three strains of *Prochlorococcus*, CCMP1986 (MED4), CCMP1375 (SS120), and CCMP2773 (MIT9313), grown under white fluorescent lamp, were investigated using picosecond (ps) time-resolved fluorescence measurements after the growth of the cells under a white fluorescent

lamp.⁵⁷ Differences in the fluorescence properties of the three strains were observed in the ps to nanosecond (ns) time-resolved fluorescence (TRF) measurements. CCMP1986, whose natural habitat is a higher-light environment, contained more DV-Chl *a* than CCMP1375 and CCMP2773, whose natural habitats are lower-light environments, and the higher-energy DV-Chl *a* in CCMP1986 was replaced by DV-Chl *b* in CCMP1375 and CCMP2773.⁵⁷

1.4 Observation of excitation energy-transfer processes

The excitation energy-transfer processes between pigments in photosynthetic organisms can be observed by detecting the fluorescence emitted by the pigments, because the fluorescence signal is detected from only the electronically excited states. Time-resolved fluorescence spectroscopy is the most useful technique to investigate the energy-transfer processes among the pigments in photosynthetic systems. By monitoring the fluorescence signals as a function of time, energy transfer is observed as the combination of time-dependent decrease and increase of fluorescence intensities. The decreased one is the energy donor while the increased one is the energy acceptor.³⁵

With the ps time-resolution, the excitation transfer process between Chl *a* are observed mainly. With the femtosecond (fs) time-resolution, on the other hand, the excitation energy transfer from Cars and Chl *b* (and Chl *c*) to Chl *a* are also detectable.³⁻¹¹ Another application is the observation of the energy-transfer between the two photosystems, PSI and PSII. The delayed fluorescence signal with long time constants detected in PSI as well as in PSII fluorescence region, after the charge recombination in PSII, may indicate the energy-transfer from PSII to PSI.⁶⁴ The delayed fluorescence observed in the 10-ns region at 77 K proceeds by the following mechanisms: When the PSII reaction center is excited, charge separation occurs. Charge recombination between the generated primary electron donor and the primary electron acceptor occurs at a given probability. After the charge recombination, an excited state is re-generated at the reaction center, and delayed fluorescence is emitted. Because of the rather long time constants, delayed fluorescence is easily distinguished from the other fluorescence signals, even from the unbound Chls. In studies of isolated PSI, the longest lifetime was determined as less than 5 ns; therefore, PSI by itself does not emit delayed fluorescence.⁶⁵ In some cases, the excited energy generated by the charge recombination in the PSII reaction center is

transferred to PSII core antenna. If this happens, fluorescence around 685 and 695 nm are observed with long lifetimes, and additional bands are detected at long wavelengths. Any long-lifetime component is not resolved in the PSI particle.⁶⁵ However, under the condition that PSII exists, long lifetime components are observed also in the PSI fluorescence region, indicating that a certain amount of PSII attaches to PSI performing a PSII-to-PSI excitation energy transfer.⁶⁴ At 77 K, energy transfer occurs from pigments with higher transition energy to those with lower transition energy (from PSII to PSI). However, at room temperature, up-hill energy transfer from PSI to PSII may be possible. Therefore, by detecting delayed fluorescence at 77 K, the mechanism by which two photosystems share excitation energy can be examined. The ps-ns time-resolved fluorescence can be observed by time-correlated single photon counting method.³⁵ The advantage of the method is the highest sensitivity that can detect even only one photon. Because of the high sensitivity, the very weak delayed fluorescence can be detected. On the other hand, a weak point of the method is the time resolution of a few ps at the shortest, because the electric response is the rate-limiting process. For the detection of the faster process, fluorescence up-conversion method can be utilized.³⁻

⁸ The time resolution of the method is almost the same to the width of the laser pulse, which is used for the gating. Fluorescence up-conversion method needs longer time than time-correlated single photon counting method for detecting the fluorescence decay curve at a wavelength, therefore it has a difficulty in observing the wide range of wavelengths in a limited time. An alternative method for the ultrafast time-resolved fluorescence measurement is optical Kerr gate.⁶⁶ It can detect the fluorescence signal with multichannel detector and it can achieve the fs time-resolution by selecting the Kerr medium without the response from the orientation relaxation process. However, the major weak point of optical Kerr gate is the large background fluorescence, which leaks from the pair of polarizers. Because of the background issue, optical Kerr gate in general has a difficulty in the detection of fluorescence signal with long average lifetime. Therefore, in the research for this thesis, time-correlated single photon counting method and fluorescence up-conversion method are utilized.

1.5 The outline of this thesis

In this research, the light harvesting functions of all the Chl *b*-containing cyanobacterium, *Prochloron*, *Prochlorothrix*, and *Prochlorococcus* were studied. In chapter 2, the excitation energy transfer between Chl *a* (and Chl *b*) in *Prochloron* and *Prochlorothrix* are discussed, utilizing the ps time-resolved fluorescence spectroscopy. The *Prochloron* cells were harboring to the four different host ascidians, and the body and habitat of the host species decided the light conditions of the cells. In chapter 3, the excitation energy transfer between DV-Chl *a*, DV-Chl *b*, and two Cars, α -carotene and zeaxanthin in the three strains of *Prochlorococcus*, grown under white fluorescent lamp are discussed, utilizing fs time-resolved fluorescence spectroscopy. In chapter 4, the light harvesting functions including excitation energy transfer between DV-Chl *a* (and DV-Chl *b*) in the three strains of *Prochlorococcus*, grown under the two different colored LEDs are discussed. One of the LED is blue-colored and is DV-Chl *b*-selective, which is comparable to the light conditions of the natural habitat of lower-light adapted strains, and the other is red-colored and is DV-Chl *a*-selective. Chapter 5 is the comprehensive discussion for the characteristics of

the Chl *b*-containing cyanobacteria inhabiting the specific light conditions, including the comparison with the other photosynthetic organisms.

Figures

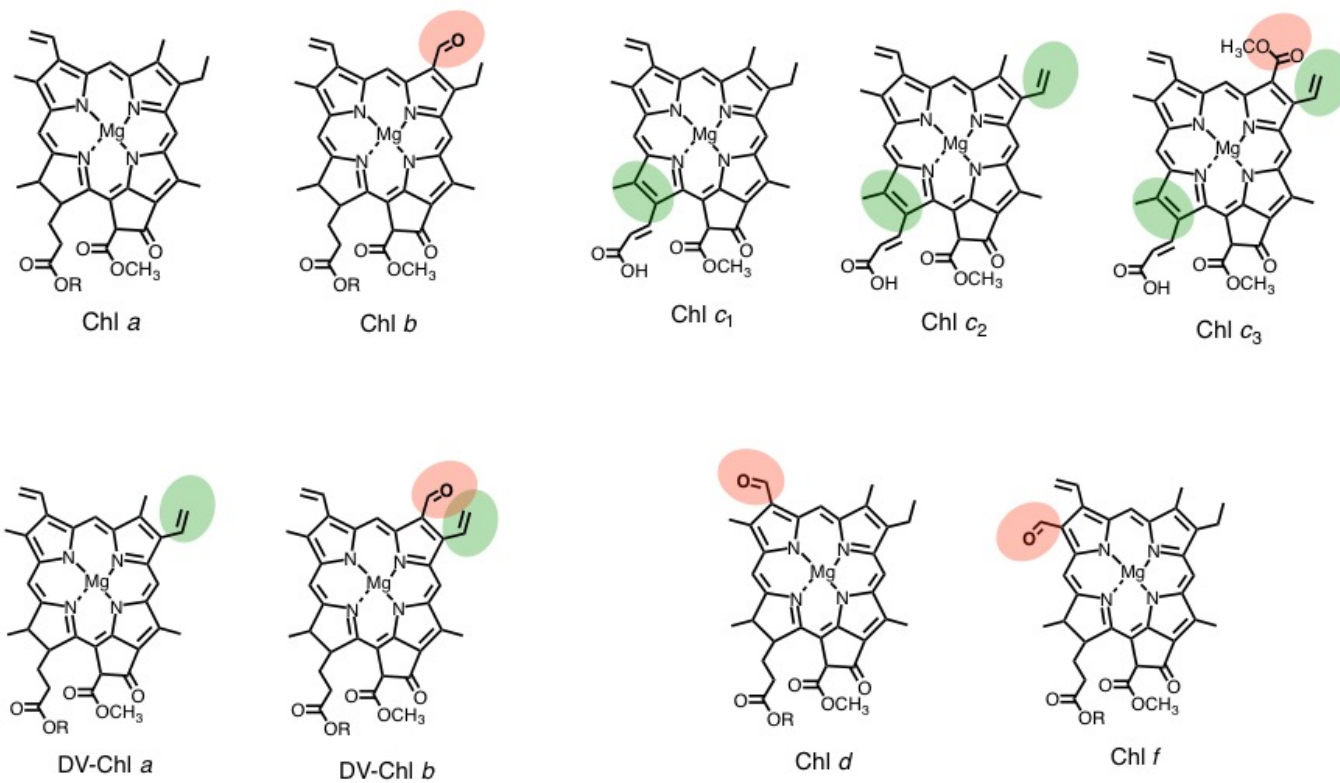


Figure 1-1. Molecular structures of the various chlorophylls found in the photosynthetic organisms.

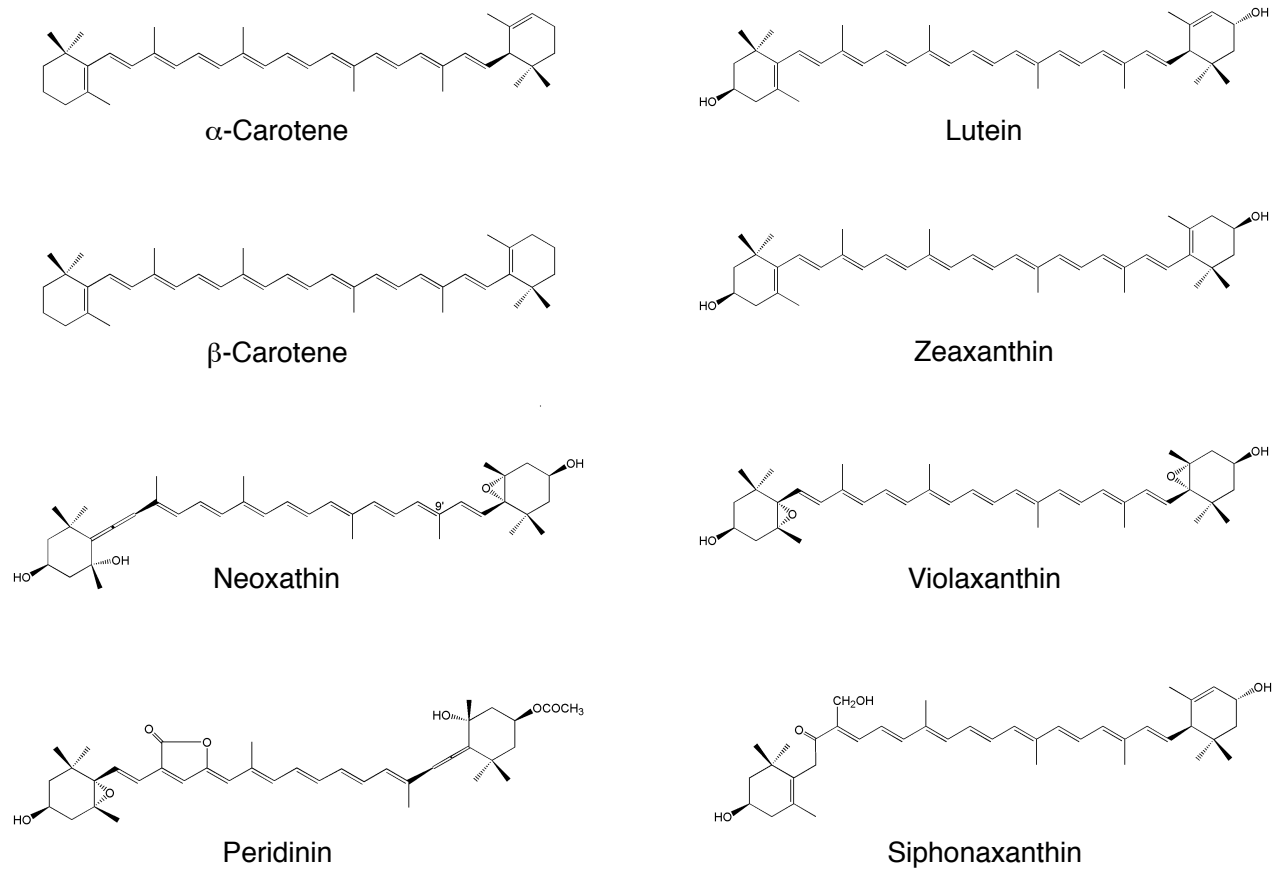


Figure 1-2. The molecular structures of some carotenoids possessed by photosynthetic organisms

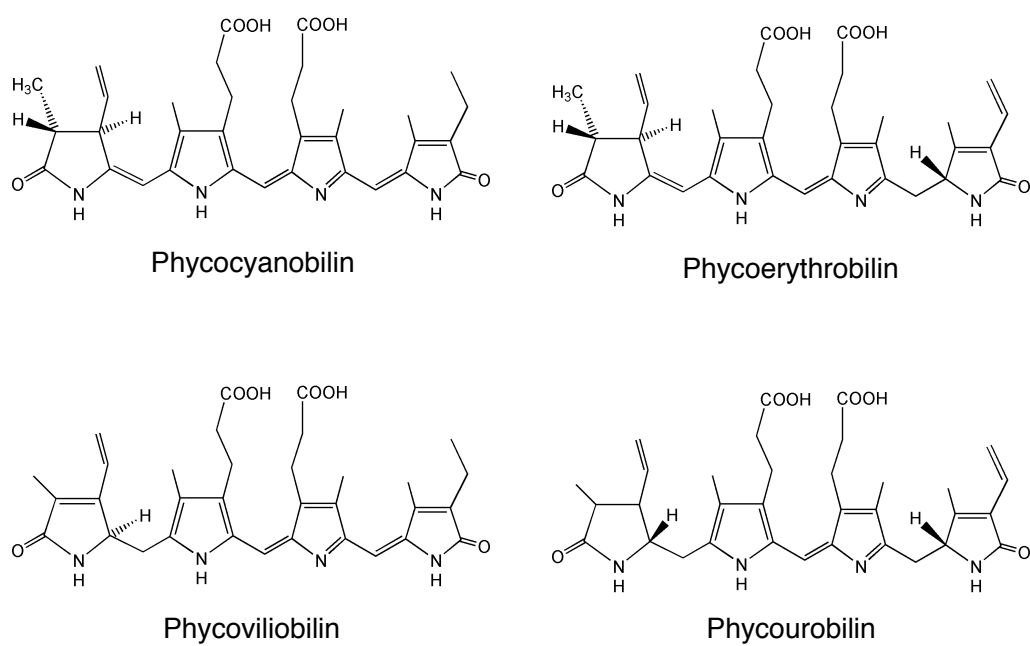


Figure 1-3. The molecular structures of phycobilins possessed by photosynthetic organisms

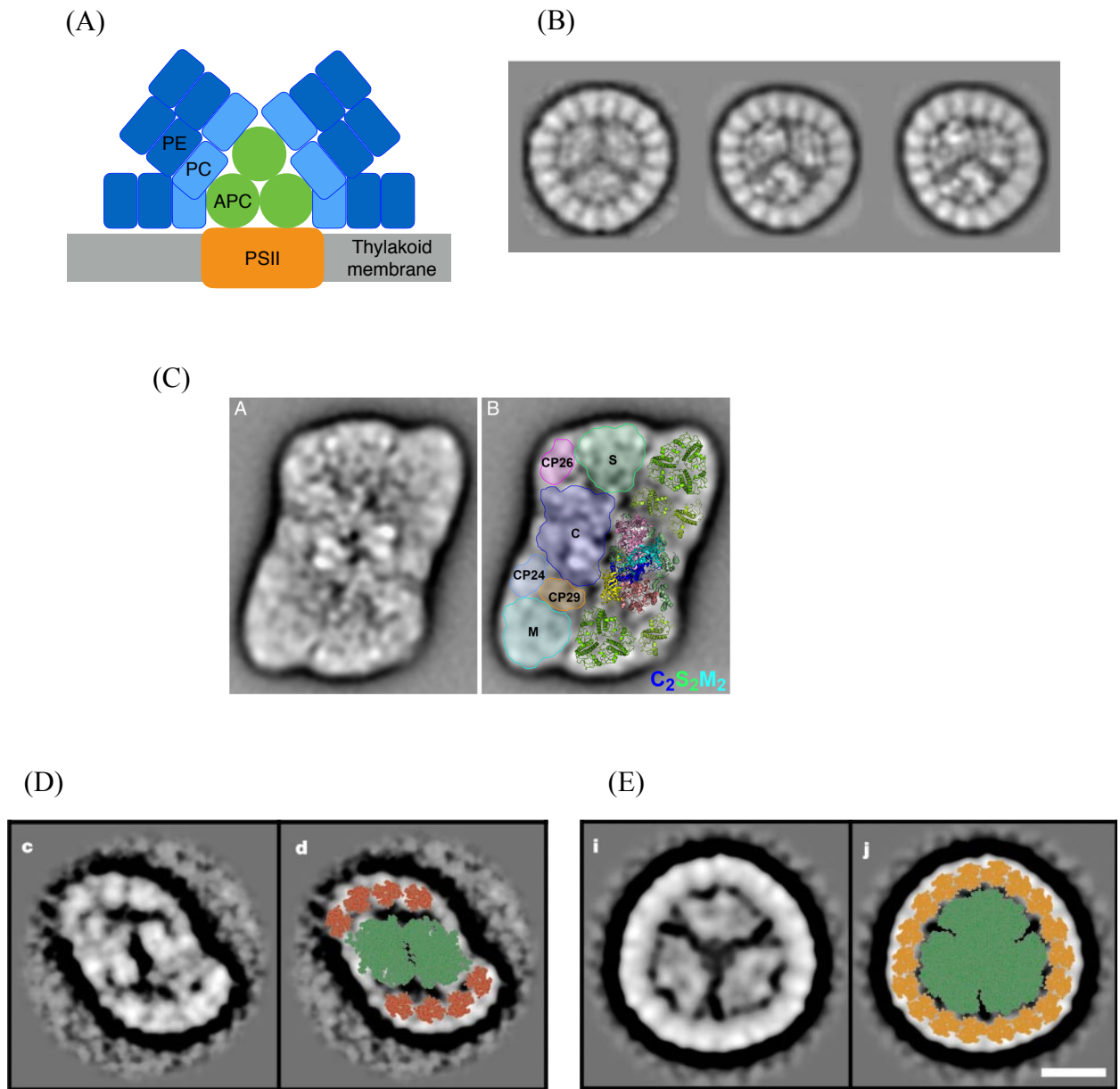


Figure 1-4. The structures of some of the peripheral antenna or the supercomplexes with photosystems; (A) The structure of phycobilisome composed of PE, PC, and APC. (B) IsiA (CP43')-PSI supercomplex from *Synechococcus* sp. PCC7942. This figure is adapted from “Boechema, E.J.; Folea, M.; Kouřil, R. Single Particle Electron

Microscopy, *Photosyn. Res.* **2009**, 102, 189–196.”. (C) LHCII-PSII supercomplex from *Arabidopsis thaliana*. This figure is adapted from “Kouřil, R.; Dekker, J.P.; Boekema, E.J. Supramolecular Organization of Photosystem II in Green Plants, *Biochim. Biophys. Acta* **2012**, 1817, 2–12.” (D) Pcb-PSII supercomplex from *Prochlorococcus* CCMP2773. (E) Pcb-PSI supercomplex from *Prochlorococcus* CCMP2773. Figures D and E are adapted from “Bibby, T.S.; Mary, I.; Nield, J.; Partensky, F.; Barber, J. Low-Light-Adapted *Prochlorococcus* Species Possess Specific Antennae for Each Photosystem, *Nature* **2003**, 424, 1051–1054.”

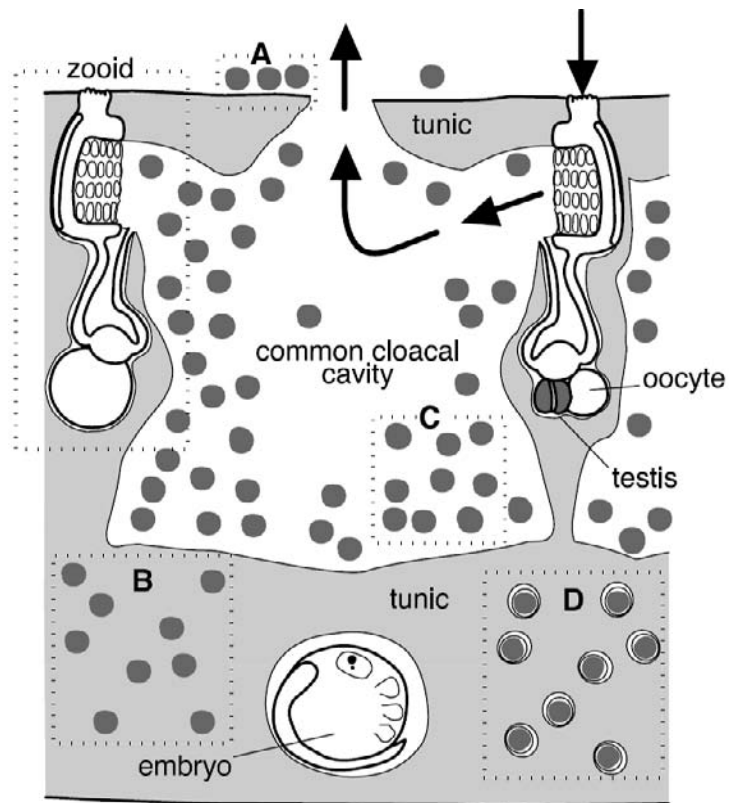


Figure 1-5. Schematic drawing of the colony harboring *Prochloron* cells. *Prochloron* cells are distributed on the colony surface, in the tunic, or in the common cloacal cavity.

This figure is adapted from “Hirose, E.; Neilan, B.A.; Schmidt, E.W.; Murakami, A. Enigmatic life and evolution of *Prochloron* and related cyanobacteria inhabiting colonial ascidians, In *Handbook on Cyanobacteria*; Gault, P.M.; Marler, H.J.; Nova Science Publishers, New York, **2009**, 161–189.”

Chapter 2. Excitation energy transfer in a symbiotic cyanobacterium *Prochloron*, occurring in coral-reef ascidians, and in a free-living cyanobacterium *Prochlorothrix*

2.1. Introduction

All of the host ascidians that established obligate symbiosis with *Prochloron*, belong to four genera of the family Didemnidae: *Didemnum*, *Trididemnum*, *Lissoclinum*, and *Diplosoma*.⁴⁴ In this study, spectral properties of *Prochloron* harbored in four species of ascidians, *Diplosoma* sp., *Trididemnum cyclops*, *Trididemnum miniatum*, and *Lissoclinum timorense* were studied. The locality of *Prochloron* cells within their host colonies varies between the host species: *Prochloron* cells are harbored in the tunic of *T. miniatum* and the cloacal cavities of the other three ascidians.^{44,67} Furthermore, *T. miniatum* hosts Group-I *Prochloron* cells, while the other four species host Group-II *Prochloron* cells (see Chap. 1). Calcareous spicules are present in the tunics of all host ascidians, except the *Diplosoma* species. Since the number of spicules depends on the ambient light within the habitat, the spicules function to protect against intense solar radiation in shallow tropical waters.⁶⁸ Although the spicules may also have an

anti-predatory function, the cytotoxic compounds contained in the *Prochloron* would be enough for the host ascidian to prevent from the predation.⁶⁹ In *T. miniatum*, the tunic contains both spicules and *Prochloron* cells, with spicule density higher in the exposed colonies than in the shaded colonies. In the colonies of *T. cyclops*, the spicules are dense in the basal part of the colonies but sparse in the upper tunic overlying the cloacal cavities in which *Prochloron* cells are distributed. In *L. timorensis*, spicule density in the upper tunic depends on the habitat of the host colony. Other than the spicules, ascidians have mycosporine-like amino acids in their colonial tunics; these mycosporine-like amino acids, which are also in *Prochloron* cells itself, protect against UV radiation damage.⁷⁰

In this study, *Prochloron in hospite* in the host ascidians and cultured *Prochlorothrix* were analyzed by means of spectroscopic measurements including ps time-resolved fluorescence spectroscopy. The light-harvesting strategy in *Prochloron in hospite* and how it varies between four ascidian hosts are discussed, with emphasis on the light conditions in ascidian habitats and the microenvironments offered by ascidians, especially their spicules.

2.2. Materials and methods

Didemnid ascidians hosting *Prochloron* were collected from the coral reef lagoon at the depths of less than one meter. *Diplosoma* sp. was collected from Yonehara (Ishigakijima Island, Japan). *T. cyclops* was collected from Bise (Okinawajima Island, Japan), while *T. miniatum* and *L. timorense* were collected from the same locality. In this thesis, *Prochloron* cells dwelling in host ascidians *in hospite* and *in situ* to avoid damage caused by the isolation processes and acidic substances in the host tissues are investigated. *Prochlorothrix hollandica* (PCC 9006) was cultured autotrophically in BG11 medium at ~ 293 K (20 °C). The culture light intensity was ca. 30 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$.

Steady-state absorption spectra were measured by a spectrometer equipped with an integrating sphere (JASCO V-650/ISVC-747) at 77 K (−196 °C). Time-resolved fluorescence (TRF) spectra were measured with a time-correlated single-photon counting system at 77 K in the same method as described previously.³³ The samples were mixed with the same volume of poly (ethylene glycol) 4000 (30 %, w/v) (Wako Pure Chemicals, Japan) to obtain homogeneous ice and were frozen in the dark.

Ti:sapphire laser (Tsunami, Spectra-Physics, USA) (80 MHz) pumped with a diode-pumped solid state laser (Millennia Xs, Spectra-Physics, USA). The excitation wavelength was 425 nm, at which all pigments (Chl *a*, Chl *b* and Cars) in *Prochloron* and *Prochlorothrix* were simultaneously excited. The time window of measurement was 10 ns with an interval of 2.4 ps/channel, or 100 ns with an interval of 24.4 ps/channel. For each sample, measurements were carried out at least 10 times for the steady-state absorption and at least 3 times for the time-resolved fluorescence. To construct the TRF spectra, fluorescence rise and decay curves were measured in the Chl *b* to Chl *a* fluorescence region (670–760 nm) with an interval of 1 nm/channel. At wavelengths below 670 nm, no signals that could be attributed to Chl *b* were observed, as is also the case for DV-Chl *b* in the high-light adapted strain of *Prochlorococcus* (CCMP1986).⁵⁷ Therefore, the time-resolved measurements of *Prochloron* and *Prochlorothrix* are discussed in terms of the signal from Chl *a* only.

2.3. Results

2.3.1 Steady-state absorption spectra of *Prochloron in hospite* and *Prochlorothrix*

Fig. 2-1 shows low-temperature (77 K) steady-state absorption spectra of *Prochloron* harbored in *Trididemnum* spp. (i.e. the complete ascidian plus *Prochloron*), together with that of *Prochlorothrix* cells cultured as a reference. *T. cyclops* and *T. miniatum* both contain calcareous spicules in their colonies. Two peaks around 440 nm and 470 nm correspond to the Soret bands of Chl *a* and Chl *b*, respectively, whereas Cars peak around 500 nm. Three peaks around 580 nm, 620 nm, and 680 nm are from the Qx band of Chl *b*, Qx band of Chl *a*, and Qy band of Chl *a*, respectively. Similar absorption bands by photosynthetic pigments are observed in all the *Prochloron* and *Prochlorothrix* cells, but large spectral differences are seen between *Prochloron* and *Prochlorothrix* cells. Especially in the spectrum of *Prochloron* in *T. cyclops*, an upward-sloping structure toward the shorter wavelength region is observed, which are in consistent with the absorption spectra of didemnid ascidians which can have symbiotic relationship with *Prochloron*.⁷⁰ It should be noted that *Prochloron* cells themselves do not exhibit the upward-sloping structure that may exceeds absorption

bands of pigments in the cells.⁴⁴ These suggest that particles in the host ascidians should scatter or absorb light in the UV to visible wavelength region. On the other hand, the upward-sloping structure is rather reduced in the spectrum of *Prochlorothrix* (compared to that of *Prochloron in hospite*). Furthermore, *Prochloron* in *T. cyclops* and in *T. miniatum* have absorption bands in 690–710 nm region that is not seen in the spectrum of *Prochlorothrix*, which suggests *Prochloron* cells utilize light in longer wavelength spectral region compared to *Prochlorothrix* cells. Spectral differences between *Prochloron* in *T. cyclops* and *Prochloron* in *T. miniatum* are also apparent; the spectrum of *Prochloron* in *T. cyclops* has a shoulder at 710 nm that is not seen in the spectrum of *Prochloron* in *T. miniatum*. Therefore, it is expected that *Prochloron* in *T. cyclops* possesses more Red Chls (low energy Chl *a*) emitting in this region than *Prochloron* in *T. miniatum*, whose purpose is to broaden the range of utilizable wavelengths when UV-visible light is scattered or absorbed by calcareous spicules.

2.3.2 Steady-state fluorescence spectra of *Prochloron in hospite* and *Prochlorothrix*

Fig. 2-2 shows steady-state fluorescence spectra of *Prochloron* harbored in the ascidian species and *Prochlorothrix*. These spectra were constructed by time-integrating the TRF spectra. Chl *a* fluorescence peaks are indicated by vertical dotted lines. Peaks at 685 nm (686 nm for *Prochlorothrix*) and 698 nm arise from Chl *a* in the core antenna complexes of photosystem (PS)II, CP43 and CP47, while the peak at 715 nm is due to Red Chl in PSI.^{52,71} Some differences in these spectra are apparent, especially in the PSI fluorescence region. *Prochloron* in *Diplosoma* sp. and *L. timorensis* show a more intense PSI signal than *Prochloron* in *T. cyclops* and *T. miniatum*. The 715 nm-band dominates in the PSI fluorescence region, and shoulders are seen around 720 nm for *Prochloron* in three of the four ascidians (the exception is *T. miniatum*, which harbors *Prochloron* in the tunic), while, for *Prochlorothrix*, the PSI signal is much smaller than PSII signal. These results suggest differences in light-harvesting strategies between *Prochloron* harbored in four different host ascidians (and also between *Prochloron* and *Prochlorothrix*), depending upon light conditions

such as the habitat of the host ascidian and the microenvironment of the ascidian colony, including calcareous spicules.

2.3.3 Time-resolved fluorescence spectra (TRFS) of *Prochloron in hospite* and *Prochlorothrix*

Fig. 2-3 shows TRF spectra of *Prochloron* harbored in the four ascidian species and *Prochlorothrix*. Intensities of these spectra are normalized relative to their maximum intensities. In the 0–5 ps timeframe, signals of Chl *a* in CP43 at 685 nm dominate all samples.⁵² After 210 ps, a signal due to Chl *a* in CP47 appears around 698 nm and increases in relative intensity. This suggests that excitation energy is transferred from CP43 to CP47 within this time region. Behavior of Chl *a* signal in the PSI region (at wavelengths longer than 700 nm) is considerably different between the samples. For *Prochloron* in *Diplosoma* sp. and *L. timorensis*, fluorescence peaks with relatively large intensities appear immediately after the excitation (0–5 ps), at 710 nm and 715 nm, respectively. On the other hand, for *Prochloron* in *T. cyclops* and *T. miniatum*, significant appearance of these peaks is delayed; *Prochloron* in *T. cyclops* fluoresces at

715 nm at 85–100 ps, whereas *Prochloron* in *T. miniatum*, at 715 nm at 570–700 ps. Apart from *Prochloron* in *T. miniatum* (which, as mentioned above, harbors *Prochloron* in the tunic), these peaks shift to 720 nm within 3 ns, and exhibit a shoulder around 730 nm. *Prochlorothrix* shows much smaller PSI signal than four *Prochloron* cells in all the timeframes.

Fluorescence signals remained in 31 ns after the excitation (Fig. 2-3), and the longest lifetimes extended to 21–26 ns (see Sec. 2.3.4 and Fig. 2-4). These lifetimes are much longer than that of a free Chl *a*; therefore, they are attributed to the delayed fluorescence originating from charge recombination occurring in the PSII reaction center.³⁵ This delayed fluorescence showed two peaks in the PSII fluorescence region at 685 nm and at 698 nm for all *Prochloron* cells, suggesting that excitation energy transfer from the reaction center to CP43 and CP47 occurs. In *Prochlorothrix*, contribution of the 698 nm band was considerably small, indicating that connection between the reaction center and CP47 is weaker. With the exception of *Prochloron* in *T. miniatum* and *Prochlorothrix*, delayed fluorescence also peaks in the PSI region around 715–720 nm, a possible indication of excitation energy transfer from PSII to PSI.⁶²

2.3.4 Fluorescence decay-associated (FDA) spectra of *Prochloron in hospite* and

Prochlorothrix

To further investigate excitation energy transfer in *Prochloron* and *Prochlorothrix*, global analysis was performed, and obtained fluorescence decay-associated (FDA) spectra.⁵⁷ Throughout the analysis procedures, spectral profiles of FDA spectra were not assumed, but analyzed the fluorescence rise and decay curves at each wavelength by fitting the sum of exponentials with common time constants as follows:

$$F(\lambda, t) = \sum_n A_n \exp(-\frac{t}{\tau_n}) \quad (1)$$

Here $F(\lambda, t)$ denotes the fluorescence decay curve at each wavelength λ , and $A_n(\lambda)$ is the amplitude of each exponential function in the curve. Positive and negative amplitudes indicate fluorescence decay and rise, respectively. A pair of positive and negative amplitudes indicates excitation energy transfer from donor with positive amplitudes to acceptor with negative amplitudes. In this analysis, the fitted decay curves of the 2.4 ps/channel interval and the 24.4 ps/channel interval of each wavelength were connected in the 2–5 ns region and treated as a single decay curve across the total time

range. The time-constants (n) were determined as reported previously,³⁵ and FDA spectra of all samples consisted of six components (Fig. 2-4).

In the FDA spectra with the fastest time constants (11-51 ps), positive amplitudes around 680 nm and negative amplitudes in the longer wavelength region were obtained in all samples (Fig. 2-4). The peak around 680 nm was assigned to Chl *a* in Pcb.⁵² While the types of Pcb in *Prochloron* is not confirmed yet, of the three kinds of Pcb in *Prochlorothrix*, PcbA and/or PcbB, which emit(s) fluorescence around 680 nm, are the most probable candidate(s); PcbC emits at 688 nm.^{29,71} PcbA and PcbB are rarely bound strongly to PSI,^{29,71} therefore, it is conceivable that these Pcb function as antenna complexes of mainly PS II, while PcbC is the main antenna of PSI. Not only PcbC, but the PSII core antenna complexes, CP43 and CP47, fluoresce in the 685–698 nm region. Profiles of FDA spectra around 688 nm depend strongly on the samples; the emission amplitudes of *Prochloron* in *T. cyclops*, *L. timorensis*, and *T. miniatum* and *Prochlorothrix* are negative, while those of *Prochloron* in *Diplosoma* sp. are positive. Within this time region, CP43 and CP47 could accept excitation energy from Pcb (especially PcbA or PcbB for *Prochlorothrix*), while Pcb (especially PcbC for

Prochlorothrix) transfers energy to the PSI complex; the former gives negative amplitudes around 688 nm, whereas the latter, positive ones. Therefore, the decay and rise kinetics are mixed in this spectral region.

In the second to fourth components of FDA spectra (the second, 150–260 ps; the third, 440–890 ps; and the fourth, 1.3–1.7 ns), positive amplitudes are obtained in 685–698 nm and in 710–730 nm regions (Fig. 2-4). At wavelengths shorter than 700 nm, positive amplitudes of CP43 (685 nm) dominate the second FDA spectra, while those of CP47 (698 nm) appear after the third FDA spectra, indicating that CP43 transfers excitation energy to CP47 in the 150–260 ps timeframe. At wavelength region longer than 700 nm, a distinct positive peak at 710 nm appears in the second FDA spectra, while the positive peaks at 715 and 720 nm occur in the third and the fourth FDA spectra. For *Prochloron* in *T. cyclops*, *Diplosoma* sp., and *L. timorensis*, a shoulder around 730 nm is also observed in the fourth FDA spectra. In the fifth FDA spectra (3.1–4.9 ns), only the signal from PSII core antenna is observed. The last FDA spectra (21–26 ns) show delayed fluorescence, in which two distinct peaks at 685 and 698 nm are recognized for all the *Prochloron* cells and only the 686 nm-band is observed for

Prochlorothrix cells. An additional peak is recognized at 715 nm for *Prochloron* in *T. cyclops*, *Diplosoma* sp., and at 720 nm for *Prochloron* in *L. timorensis*. This indicates an excitation energy transfer pathway from PSII to PSI in these time frames.⁶⁴ For *Prochloron* in *T. miniatum*, the relative intensity of delayed fluorescence in the PSI region is negligibly small, suggesting that PSII and PSI are not strongly energetically bound in this sample. For *Prochlorothrix*, relative intensities in the PSI region to those in the PSII region are smaller than those for *Prochloron* after the third FDA spectra.

2.4. Discussion

The TRF and FDA spectra of *Prochloron* in four different ascidians indicate that the composition of the spectral components and the efficiency of excitation energy transfer between antenna proteins in *Prochloron* change with host species. Firstly, in FDA spectra, the time constants of excitation energy transfer from the peripheral antenna Pcb to PSII and PSI can be classified into two groups. For *Prochloron* in *T. cyclops* and *Diplosoma* sp., the excitation energy transfer occurs with faster time constants (11–18 ps), while it is slower (50–51 ps) for *Prochloron* in *T. miniatum* and *L.*

timorensis (Fig. 2-4). Differences between these samples might be due to the various light conditions of the host ascidians' habitat. In the present study, the collected colonies of *T. miniatum* and *L. timorensis* inhabited sites that are strongly exposed to light, while the colonies of *T. cyclops* and *Diplosoma* sp. inhabited shaded sites with lower light exposure. It is therefore suggested that *Prochloron* changes the excitation energy transfer efficiency among its antenna proteins depending upon the light conditions of the habitat of host ascidians, and that *Prochloron* in shaded ascidians possess the light harvesting system with higher energy transfer efficiency. These are supported by the fact that the first FDA spectra of *Prochlorothrix* (50 ps), which is not shaded by a body of ascidian, shows almost the same behavior as *Prochloron* in *T. miniatum*, which habitates under the shaded environment. Furthermore, in the fifth FDA spectra, the amplitudes are smaller for *Prochloron* in *T. miniatum* and *L. timorensis* than for *Prochloron* in the other host species. This suggests that excitation energy is quenched within antenna Pcb by the presence of excess excitation energy under sunny conditions. In this sense, smaller amplitude in the fifth FDAS in *Prochlorothrix* than

those of *Prochloron* in *T. miniatum* and *L. timorensis* suggests that *Prochlorothrix* is under relatively higher-light adapted condition.

Differences between the four *Prochloron* cells regarding the Red Chls in PSI were also apparent. All the cells show fluorescence of the PSI Chls at 710 nm, 715 nm, and 720nm (Figs. 2-3 and 2-4). In addition, *Prochloron* in *T. cyclops*, *Diplosoma* sp., and *L. timorensis* fluoresce around 730 nm. As discussed above, *T. cyclops* and *Diplosoma* sp. inhabit shaded areas; therefore, *Prochloron* in these two ascidians require an efficient light harvesting system. Not only the energy transfer efficiency between the pigments, but the presence of Red Chls in these ascidians can broaden their utilizable wavelength region. By contrast, *L. timorensis* (and *T. miniatum*) was under sunny condition, in which the efficiency of the light-harvesting system is less stringent. The presence of Red Chls in *Prochloron* in *L. timorensis* is difficult to explain based on the hosts' habitat, but the ascidian tunic and calcareous spicules both absorb light of shorter wavelengths, as the absorption spectra show. In *T. miniatum*, *Prochloron* cells and spicules reside together within the tunic, which may partially enable the *Prochloron* cells to absorb sunlight without being shaded by spicules. On the other hand, in *L.*

timorensis, *Prochloron* cells are in the cloacal cavity, and therefore receive light through calcareous spicules. Therefore, it is likely that by possessing the Red Chls, *Prochloron* in *L. timorensis* can utilize light energy of longer wavelengths under appropriate conditions. Moreover, significant self-shading effect by the cells is possible, because the *Prochloron* cells are at a very high density in the cloacal cavities. The difference of subcellular structure in *Prochloron* cells may also cause the presence or absence of Red Chls; *Prochloron* cells in *T. miniatum* are belonged to Group-I (the central region of the cell is filled with small ‘vacuoles’), while those in *T. cyclops*, *Diplosoma* sp., and *L. timorensis* are Group-II (there is a large ‘vacuole’ in the cell).^{44,67} The PSI Chl signal of *Prochlorothrix*, are much smaller than that of *Prochloron*. Therefore, it seems that *Prochlorothrix* utilizes shorter wavelength region and has relatively high-light-adapted nature than *Prochloron*.

In the latest time region of TRF spectra and the sixth component of FDA spectra, all *Prochloron* cells show definitive signals at 685 nm and 698 nm (Figs. 2-3 and 2-4), indicating that the charge recombination in the PSII reaction center occurs in all the cells, while *Prochlorothrix* shows the definitive signal only at 685 nm with much

smaller one at 698 nm. In addition, clear delayed fluorescence in the PSI region occurs in the sixth FDA spectra of *Prochloron* in *T. cyclops* and *Diplosoma* sp. (peaking at 715 nm), and in *L. timorensis* (peaking at 720 nm). These results suggest that (1) excitation energy caused by charge recombination is efficiently transferred to PSI complexes through PSII core antenna (indicating that PSII and PSI are energetically bound), and (2) the final energy trap following PSII-to-PSI excitation energy transfer depends on the environment of the habitat of the cells. The reason why the excitation energy transfer between PSII and PSI is possible is that *Prochloron* lacks some of the Pcb antenna in the Pcb-PSI supercomplex.⁵⁵ Another Pcb-containing cyanobacterium, *Prochlorococcus*, is reported to have a complete antenna ring complex around PSI, which may prevent the direct excitation energy transfer between PSII and PSI.^{54,95}

In summary, *Prochloron* in *T. cyclops* and *Diplosoma* sp. possess relatively efficient light-harvesting systems, mediated by the higher efficiency of excitation energy transfer from PSII to PSI, which are energetically associated. The light harvesting systems of *Prochloron* in the other two ascidians, *T. miniatum* and *L. timorensis*, are less efficient. *Prochloron* in shaded habitats might develop effective

light-harvesting systems via association between two photosystems, in order to enhance the excitation energy transfer. However, *Prochloron* in *L. timorensis* also clearly exhibited PSII-to-PSI excitation energy transfer, though this *Prochloron in hospite* inhabits sunny areas. From the tendencies in the other cells, it is recognized that *Prochloron in hospite* adjust their light harvesting systems according to the light conditions of their habitats. In *L. timorensis*, “shade” effects from spicules of the host ascidian that scatter and/or absorb light of shorter wavelengths is indicated. *Prochloron* in *L. timorensis* might have adaptive strategies to utilize longer-wavelength light by producing more Red Chls and by maximizing the efficiency of the PSII-to-PSI excitation energy transfer, because the host ascidian has been equipped with spicules as protection against intense sunlight. On the other hand, *Prochlorothrix* shows some behaviors similar to *Prochloron*, especially that in *T. minutum*, but *Prochlorothrix* has even less PSI Chl and does not utilize lights in longer wavelength region of photosynthetic active radiation, compared to *Prochloron*. Therefore, the two Chl *b*-containing cyanobacteria, symbiotic *Prochloron* and free-living *Prochlorothrix*, must

have adapted to light environment in their respective habitat through different adaptive strategy.

2.5 Publication statement

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Figures

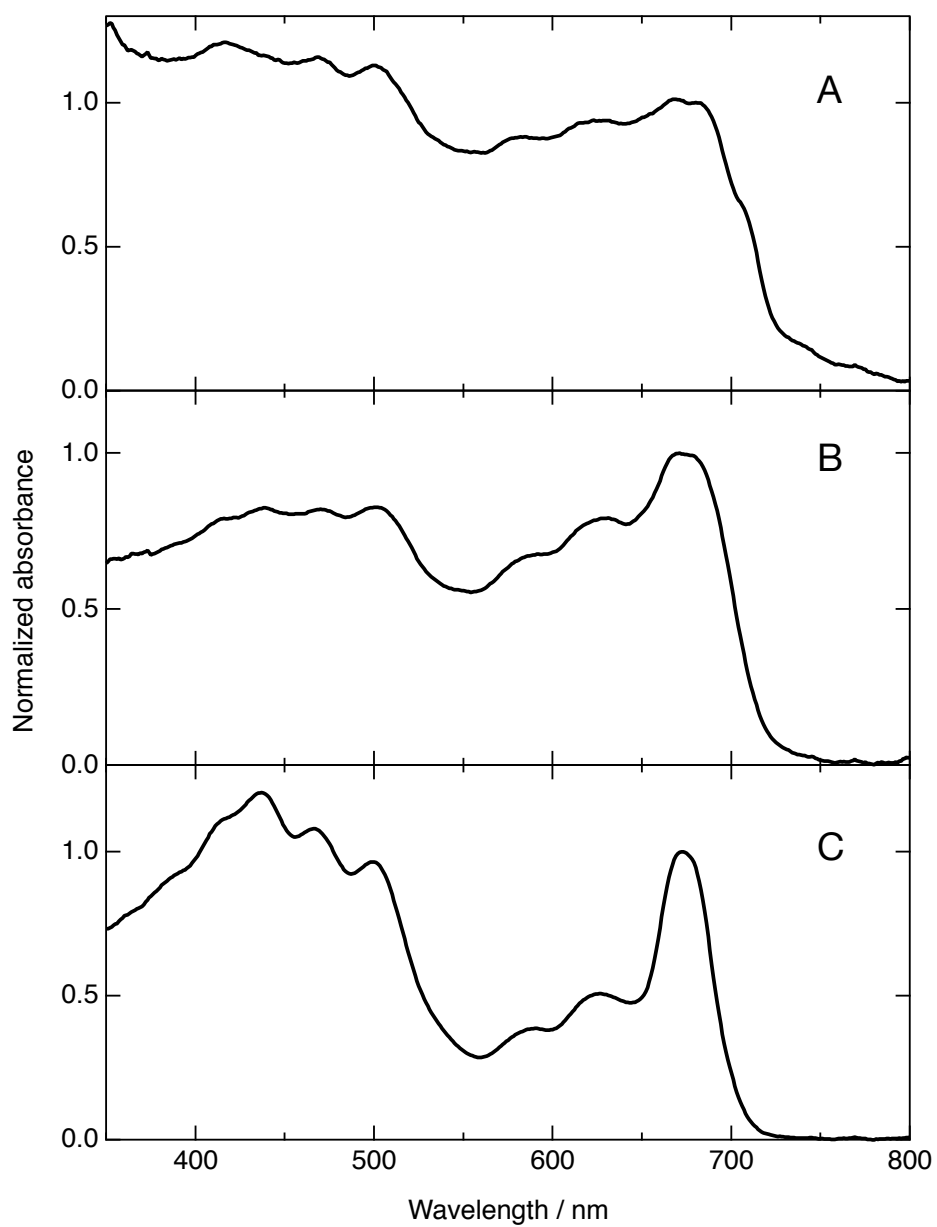


Figure 2-1. Absorption spectra of *Prochloron in hospite* and *Prochlorothrix* observed at 77 K: (A) *Prochloron* in *Trididemnum cyclops*, (B) *Prochloron* in *Trididemnum miniatum*, and (C) *Prochlorothrix*.

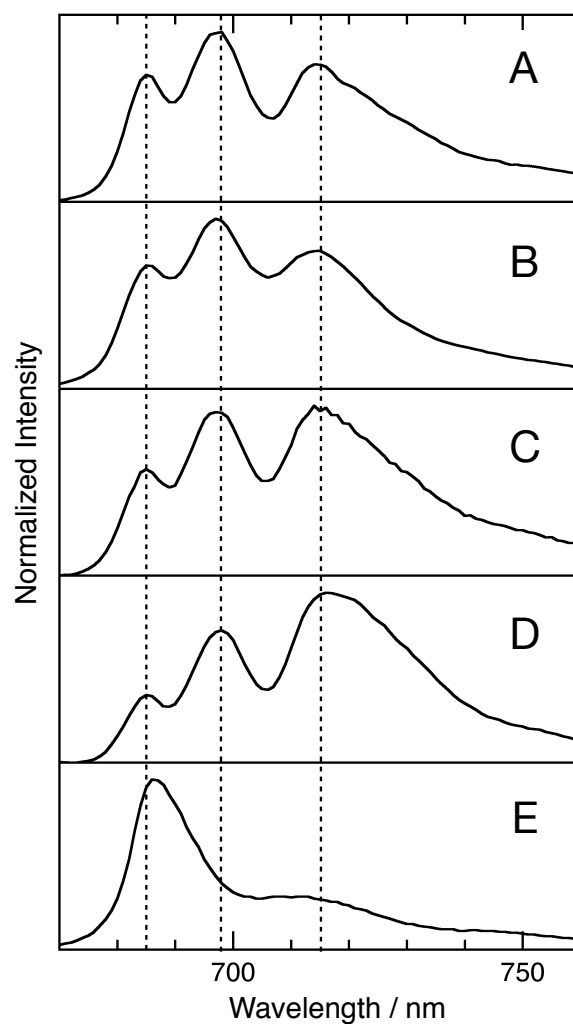


Figure 2-2. Steady-state fluorescence spectra of *Prochloron* in *hospite* and *Prochlorothrix*: (A) *Prochloron* in *Trididemnum cyclops*, (B) *Prochloron* in *Trididemnum miniatum*, (C) *Prochloron* in *Diplosoma* sp., (D) *Prochloron* in *Lissoclinum timorense*, and (E) *Prochlorothrix* observed at 77 K. Excitation wavelength is 425 nm. Spectra are normalized relative to their maximum intensities. Vertical dotted lines indicate 685 nm (PSII), 698 nm (PSII), and 715 nm (PSI).

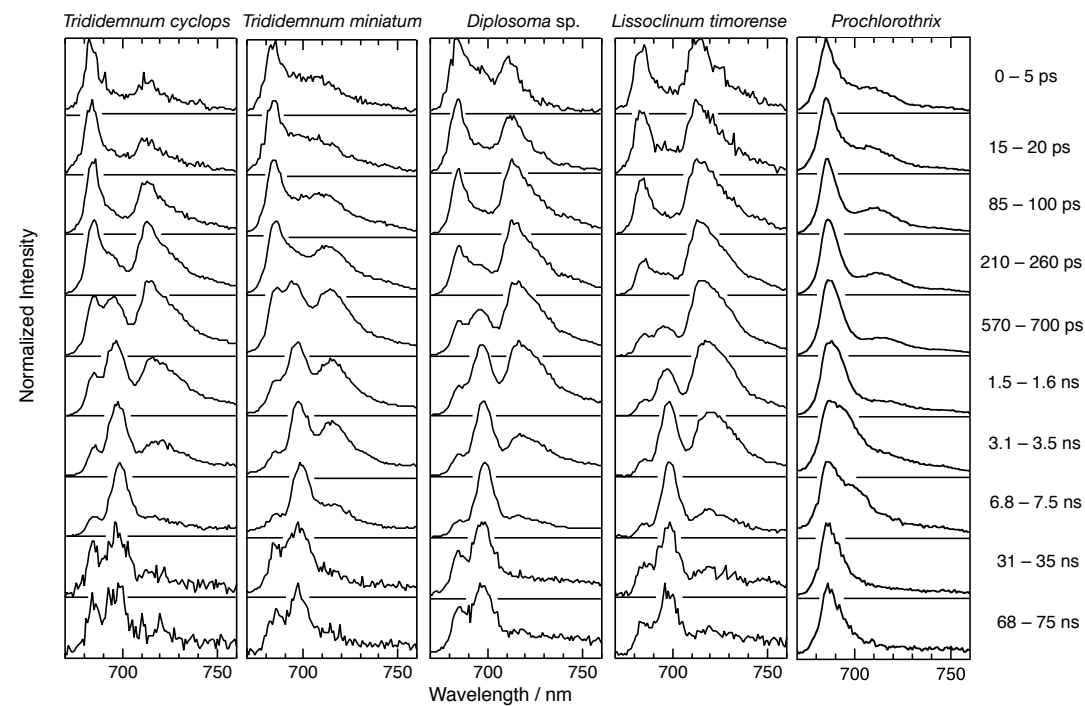


Figure 2-3. Time-resolved fluorescence (TRF) spectra of *Prochloron* in *Trididemnum cyclops*, *Prochloron* in *Trididemnum miniatum*, *Prochloron* in *Diplosoma* sp., *Prochloron* in *Lissoclinum timorense*, and *Prochlorothrix* at 77 K. Excitation wavelength is 425 nm. Spectra are normalized relative to their maximum intensities.

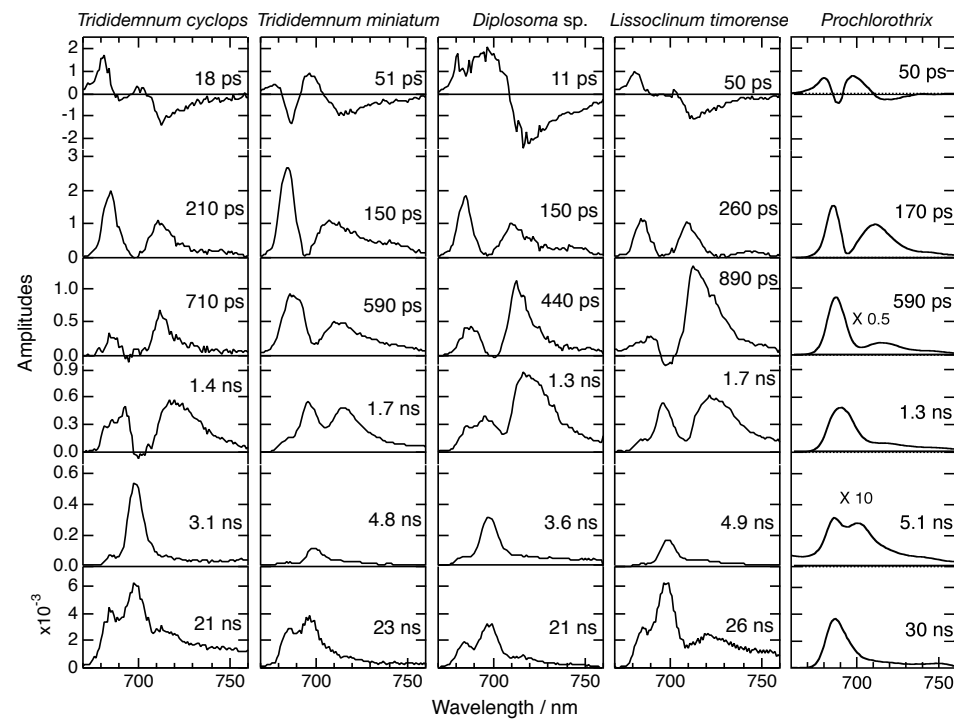


Figure 2-4. Fluorescence decay-associated (FDA) spectra of *Prochloron* in *Trididemnum cyclops*, *Prochloron* in *Trididemnum miniatum*, *Prochloron* in *Diplosoma* sp., *Prochloron* in *Lissoclinum timorense*, and *Prochlorothrix* observed at 77 K. These spectra are normalized relative to the intensity at 710 nm in the second components (150–260 ps).

Chapter 3. Comparative analysis of ultrafast excitation energy-transfer pathways in three strains of divinyl chlorophyll *a/b*-containing cyanobacterium *Prochlorococcus*

3.1. Introduction

Prochlorococcus is an exceptional organism that accumulates DV-Chls instead of the monovinyl Chls found in most photosynthetic organisms (including cyanobacteria, algae, and plants).⁵⁷ The energy levels of DV-Chl *a* and DV-Chl *b* differ from those of (monovinyl) Chl *a* and Chl *b*, respectively; this difference is especially clear in the Soret band absorption.⁷² The Soret band absorption of DV-Chl *b* peaks in the longest wavelength region among all Chls; consequently, *Prochlorococcus* can use the limited blue–green light in the lower euphotic zone (depth = 50–150 m) of tropical and subtropical oceans.⁷³

In this study, the light-harvesting functions of the photosynthetic pigments DV-Chl *a*, DV-Chl *b*, and two types of Cars, α -carotene and zeaxanthin, in intact cells of *Prochlorococcus* are investigated. For this purpose, ultrafast time-resolved fluorescence measurements and steady-state measurements are made at physiological

temperature (293 K).⁵⁸ The light-harvesting functions of the strains are compared, focusing on the excitation energy transfers between their pigments, which occur in the fs to ps time domains.

3.2. Materials and methods

3.2.1 Culture condition

Three strains of *Prochlorococcus*, CCMP1986 (MED4), CCMP1375 (SS120), and CCMP2773 (MIT9313), were cultured autotrophically in PCR-S11 medium at 293 K (20 °C). The fluorescence lamp was illuminated in the 12 h light-12 h dark regime and the light intensities were adjusted according to the strains: 40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for the high-light adapted strain (CCMP1986), and 4 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for the low-light adapted strains (CCMP1375 and CCMP2773), as reported elsewhere.⁵⁷

3.2.2 Spectroscopy

Steady-state absorption and fluorescence spectra were measured by a spectrometer (V-650, JASCO, Japan) and a fluorometer (FP-6600, JASCO, Japan),

respectively. The excitation wavelength of the fluorescence measurement was 425 nm. Time-resolved fluorescence was measured with a fs fluorescence up-conversion method, as described previously.⁷⁴ The excitation was triggered by the second harmonic (425 nm) of a Ti:sapphire laser (Tsunami, Spectra-Physics, USA) (80 MHz) pumped with a diode-pumped solid state laser (Millennia Xs, Spectra-Physics, USA). To avoid polarization effects, the angle between the polarizations of the excitation and probe beams was set to the magic angle by a $\lambda/2$ plate. The measurement time window was 10 ps, and the time interval for the decay curve acquisition was 33 fs/channel. The instrumental response function had a pulse width of 180 fs (FWHM (full width at half maximum)). Fluorescence decay curves were measured between 650 and 700 nm at 5-nm intervals. The decay curves were also measured at the peak of the steady-state fluorescence spectra (682 nm). All measurements were carried out at 293 K. Analytical calculations were carried out by Igor Pro (ver. 6, WaveMetrics, Inc.). FDA spectra were constructed by global analysis (see sec. 3.3.4 for details). To construct the TRF spectra, the relative intensities between the decay curves at different wavelengths were adjusted so that the FDA spectra with the longest time constants (> 20 ps) overlapped the

steady-state fluorescence spectra in the shapes.

3.3. Results

3.3.1 Steady-state absorption spectra

Steady-state absorption spectra at 293 K are shown in Fig. 3-1a. CCMP1986 shows a DV-Chl *a* Qy band at 674 nm with a vibrational band around 620 nm, DV-Chl *a* Soret bands around 445 nm with a shoulder around 430 nm, and the S₂←S₀ absorption bands of Cars (α -carotene and zeaxanthin) around 480–490 nm. In *Prochlorococcus* CCMP1986, the Car band is mainly contributed by the shoulder around 490 nm, which is attributed to zeaxanthin.⁴ CCMP1375 and CCMP2773 both show clear additional bands of DV-Chl *b* around 659 nm (Qy) and 479 nm (Soret). In the Qy region, it is observed that the DV-Chl *a* signal is weaker in CCMP2773 than in CCMP1375, indicating that CCMP2773 has a higher DV-Chl *b*/DV-Chl *a* ratio than CCMP1375. The strong DV-Chl *b* Soret band obscures the Car band in CCMP1375 and CCMP2773; however, according to one report, the α -carotene and zeaxanthin contents are comparable between these two strains.⁵⁷ In acetone solutions, DV-Chl *a* shows bands at

380, 416, 438, 540, 580, 617, and 665 nm, while DV-Chl *b* at 389, 439, 468, 545, 600, and 651 nm.⁷⁵ The peaks should shift in cells because of pigment-protein interactions or pigment-pigment interactions.

3.3.2 Steady-state fluorescence spectra

The steady-state fluorescence spectra of the three strains of *Prochlorococcus* are shown in Fig. 3-1b. All of the strains exhibit a main fluorescence peak at 682 nm with a weak vibrational band around 730 nm, because of fluorescence from DV-Chl *a*. The fluorescence from DV-Chl *b* appears as the enhancement at wavelengths shorter than the DV-Chl *a* band. CCMP1986 exhibits different characteristics from the other strains; its spectrum lacks the DV-Chl *b* signal around 660 nm, and shows broader fluorescence of DV-Chl *a*. In CCMP1375 and CCMP2773, the signal from higher-energy DV-Chl *a* is likely replaced by DV-Chl *b*.

3.3.3 Time-resolved fluorescence spectra

Fig. 3-2 shows the normalized TRF spectra of the three strains (circles), and the results of the spectral component analysis (for details, see sec. 3.3.5). Signals at wavelengths shorter than 675 nm are significant in the early times for all three strains. The relative intensities of these signals decrease in the later times, while signals around 682 nm dominate in the later times. The time evolutions of the spectral shapes significantly differ between the strains. The TRF spectra of CCMP2773 show significantly different shape characteristics from the other two strains, especially in the earlier times. In CCMP1375 and CCMP2773, the shorter-wavelength fluorescence signal remains in the later times, because of thermal equilibrium between the pigments.⁵⁷ In CCMP1986, shorter-wavelength signals appear only at times earlier than 1 ps, because the DV-Chl *b* content is much smaller in CCMP1986 than in the other strains. It was reported that the relative amount of DV-Chl *b* to DV-Chl *a* in CCMP1986 was as small as from one fifth to one tenth of the other two strains.⁵⁷ The shorter-wavelength signals in the early times of CCMP1986 should be contributed by S₂ fluorescence from the Cars.

3.3.4 Fluorescence decay-associated spectra

To understand the energy transfer pathways, a global analysis of the measured fluorescence kinetics data was conducted (see sec. 2.3.4). The FDA spectra obtained by the global analysis are presented in Fig. 3-3. The measured fluorescence rise and decay curves were analyzed by the convolution of the exponentials (Eq. 1 in sec. 2.3.4) and the Gaussian function with a width of 180 fs (FWHM).

The resulting FDA spectra reveal the excitation energy transfer pathways. In each FDA spectrum, negative and positive regions are found, which correspond to fluorescence rise and decay signals, respectively. The FDA spectra were constructed from four lifetime components ($n = 1, 2, 3$, and 4), because a three-component analysis was insufficient to simultaneously represent all fluorescence rise and decay curves, while a five-component analysis provided spectra that contradict to each other with the near time constants. The time constants for the DV-Chl *a*-rich strain (CCMP1986) were 140 or 110 fs, 480 fs, 6.1 ps, and longer than 20 ps (>20 ps). At shorter wavelength regions, the fluorescence kinetics of this strain show intense decay components attributed to Car S_2 decay, while at the longer wavelengths, a rise component due to

internal conversion in DV-Chl appears. For that reason, the first FDA spectra of CCMP1986 was analyzed separately with two different lifetimes (140 fs at 650, 655, and 660 nm, versus 110 fs at wavelengths longer than 665 nm). The global analysis of CCMP1375 yielded FDA spectra with time constants of 110 fs, 860 fs, 8.1 ps and >20 ps, on the other hand, for CCMP2773, the time constants were 120 fs, 830 fs, 5.0 ps and >20 ps. In both of these strains (CCMP1375 and CCMP2773), the rise component from the internal conversion in DV-Chl dominated even in the shorter wavelength region, probably reflecting the relatively high DV-Chl *b* content in these strains. For all strains, the fourth lifetime value (>20 ps) was based on previously reported ps time-resolved measurements,⁵⁷ and the shapes of the FDA spectra were corrected by adjusting the relative intensities between the measured fluorescence decay curves, such that the FDA spectra with the longest time constants (>20 ps) coincided the steady-state fluorescence spectra.

From the FDA spectra, the overall dynamics of the pigments in *Prochlorococcus* is derived as follows: internal conversions of DV-Chl (within ~110 fs) is followed by fast excitation energy transfer among the pigments (480–860 fs), slow

energy transfer and energy equilibration among the pigments (5.0–8.1 ps), and finally by relaxation after the equilibrium (> 20 ps).

The FDA spectrum with the shortest lifetime of CCMP1986 reveals significant decay of Cars (140 fs) in the shorter wavelength region. The fluorescence decay was also measured at 560 nm, yielding a lifetime of 150 fs for this strain. This lifetime is almost identical to the lifetimes of S₂ of lutein in acetonitrile (145 fs) and 9'-*cis* neoxanthin in *Arabidopsis* LHCII (145 fs),^{5,6} indicating that energy transfer from the S₂ state of Cars is inefficient. In contrast, the decay lifetime at 560 nm was 120 fs in both CCMP1375 and CCMP2773, indicating that the Cars in these strains energetically couple with DV-Chls.

3.3.5 Fluorescence spectral components decomposition and its time evolution

Although the FDA spectra reveal the averaged excitation energy transfer pathways, they cannot elucidate the details of the relaxation dynamics with small amplitude changes. In order to see this, the steady-state and time-resolved fluorescence spectra were analyzed simultaneously by an alternative two-step process.⁷⁶

First, the measured fluorescence spectra (both steady-state and time-resolved fluorescence) was analyzed with common Gaussian spectral components; second, the time-evolution of the resolved spectral components was analyzed. In this process, the peak positions of the spectral components were common to all time frames, and to all strains; thus, the three strains were analyzed with common spectral components. To satisfactorily analyze the spectra with minimum number of components, the analysis by six components (F648, F660, F672, F682, F687, and F698) for the TRF spectra was selected, because five components were not enough to represent the significant behaviors, especially in early times of CCMP2773. F648 and F660 could be assigned as the signals from DV-Chl *b*, while F672, F682, F687, and F698 as those from DV-Chl *a*, based on the report on a green algae *Bryopsis maxima*, in which the numbers of components for Chl *b* and Chl *a* are two and four, respectively.⁷⁷ The peak wavelengths of the components fitted to the TRF spectra are listed in Table 3-1. Additional components were needed to fit wavelengths longer than 700 nm in the steady-state spectra. The blue-edge and red-edge components, F648 and F698, respectively, exhibited broader bandwidths than the intermediate components, F660–F687 (Fig. 3-2).

The F648 component should be contributed by both bands of DV-Chl *b* and the S₂ state of the Cars. Although the spectra of the Car S₂ should peak at wavelengths shorter than 648 nm, their bandwidths are sufficiently broad to cover the F648 peak.⁷⁸ F698 might also be affected by minor components such as the lower lying vibrational bands of DV-Chl *a*.

After the spectral decomposition, the time evolutions of the spectral components were analyzed by sums of exponentials. In this analysis, the instrumental response is convoluted as a Gaussian function with a width of 180 fs (FWHM). Different from the global analysis, the time evolutions of the spectral components were analyzed individually, so common time constants were not assigned to the components. The time evolutions of the spectral components are shown in Fig. 3-4, and the analyzed time constants and amplitudes are listed in Table 3-1. Like the global analysis, this analysis required four lifetimes, but the lifetime values may differ between the evolution and global analysis.

3.4. Discussion

3.4.1 Clarification of the energy transfer pathways in *Prochlorococcus*

The FDA spectra reveal differences in the spectral shapes between the three strains. The characteristics of the higher-light adapted strain CCMP1986 is firstly discussed. CCMP1986 contains relatively less DV-Chl *b* than the other two strains; therefore, it was expected that Car decay signals (140 fs) would emerge in shorter wavelength region. Additionally, short-wavelength decay components are also observed in the second (480 fs) and the third (6.1 ps) FDA spectra, which correspond to energy transfer pathways among pigments of CCMP1986. However, because the time constants (480 fs and 6.1 ps) are too long to be assigned to Car S₂ decay, these components should mainly arise from DV-Chl *b*. Although CCMP1986 contains limited DV-Chl *b*, this DV-Chl *b* may efficiently transfer excitation energy to DV-Chl *a*. The decay amplitude seems smaller than the rise amplitude in the second and third FDA spectra, but this might be explained by the fact that the absorption coefficient of DV-Chl *a* is larger than that of DV-Chl *b*.⁷³ This indicates that the rise signals in the DV-Chl *a* band region over 670 nm are contributed mainly by energy transfer from DV-Chl *b*.

The FDA spectra of the lower-light adapted strains, CCMP1375 and CCMP2773, show different characteristics from that of CCMP1986. The time constants of the second FDA spectra, which shows energy transfer pathways of CCMP1375 (860 fs) and CCMP2773 (830 fs), are longer than that of CCMP1986 (480 fs), possibly because the lower-light adapted strains, which must efficiently collect the limited light in deeper euphotic zones in tropical ocean, contain more DV-Chl *b* than CCMP1986. In the second FDA spectra, the broad negative band of DV-Chl *a* is contributed not only by excitation energy transfer from DV-Chl *b* but also by energy transfer from the S_1 state of the Cars to the lower lying state of DV-Chl *a*. In the excitation spectra of *Prochlorococcus*, the DV-Chl *a* fluorescence band exhibited clear Car signals, indicating efficient energy transfer from the Cars to DV-Chl *a*.⁵⁷ Meanwhile, judging from the S_2 lifetime analyzed in the present work, the energy transfer efficiency from the Car S_2 state is too low to dominate the signal. This suggests an energy transfer pathway from Car S_1 to DV-Chl *a*. Dotted and broken lines at 676 nm and 687 nm, respectively, are superimposed on all FDA spectra in Fig. 3-3. The former corresponds to the energy level of the S_1 state of α -carotene;⁷⁹ the latter to the S_1 state of

zeaxanthin.⁸⁰ S_1 – S_0 transitions in Cars are usually forbidden and dark, but excitation energy transfer from Cars (S_1) to Chls (Qy, S_1) may occur through quadrupole–dipole interactions.⁸¹ Therefore, in the lower-light adapted strains, CCMP1375 and CCMP2773, Cars (especially α -carotene) probably work as the efficient antenna pigments. Zeaxanthin can partially contribute to light harvesting, but its S_1 state is lower than that of α -carotene; moreover, the primary role of zeaxanthin is to dissipate excess energy in xanthophyll cycles.⁸² By contrast, the third FDA spectrum (5.0 ps) of CCMP2773 displays significant negative signals at 680 and 682 nm, indicating energy transfer to DV-Chl *a*. However, the corresponding energy donor is not necessarily clear because the third FDA spectrum is flat, except for the negative signals at 680 and 682 nm and a positive DV-Chl *b* signal at 660 nm. In the third FDA spectra, which corresponds to slower energy transfer, the negative amplitude around 670 nm is less evident in CCMP1375 than in CCMP1986, indicating that DV-Chl *a* (with a fluorescence peak at 670 nm) accepts excitation energy more efficiently in CCMP1375 than in CCMP1986.

The time evolution of the analyzed fluorescence spectral components is discussed next (Figs. 3-2 and 3-4, and Table 3-1). In CCMP1986, the first components

of F672 (100 fs), F682 (120 fs), F687 (110 fs), and F698 (130 fs) relate to internal conversion of DV-Chl *a* from higher excited states to the Q_y states: therefore, the amplitudes of these four spectral components are negative for the lifetimes of ~100 fs.⁵

The amplitudes of two spectral components (F648 and F660) become positive for their first lifetimes (160 and 130 fs), because of the consistence with the decay signal of the Car S₂ state in the FDA spectra. The first decays of F648 and F660 show large relative amplitudes (0.77 and 0.51, respectively), indicating that F648 and F660 are dominated by the decay signals of Cars. The second lifetime of the F687 component shows a fast rise of 380 fs; in contrast, the second lifetimes of the other spectral components are 650–900 fs. This indicates that both DV-Chl *b* and the Car S₁ state are related in the energy transfer pathway to DV-Chl *a* (F687). Apart from F687, excitation energy is transferred between DV-Chl *b* and DV-Chl *a* in the second and third lifetimes, as evidenced from the decay and rise components (with lifetimes of 650–900 fs and 4.6–6.5 ps, respectively). The time constants of 650–900 fs are comparable with those of energy transfer from Chl *b* to Chl *a* in LHC II of the green alga *Codium fragile*¹³ and the higher plant *Arabidopsis thaliana*,⁶ indicating that DV-Chl *b* also functions as an

efficient antenna pigment, in spite of its limited content in the higher-light adapted strain. F687 shows a large negative amplitude with a lifetime of 5.6 ps, while F672 and F682 show smaller negative amplitudes with corresponding lifetimes of 6.0 and 6.2 ps. This indicates that among the molecules of DV-Chl *a* having different energy levels, F687 is the main acceptor of the excitation energy from Car S_1 states. However, as shown in Fig. 3-4, F687 has smaller amplitude than the other components. Therefore, the F687 component, which mediates energy transfer in CCMP2773 (see below), makes no significant contribution to energy transfer in the higher-light adapted strain, CCMP1986.

For CCMP1375, which contains more DV-Chl *b* than CCMP1986, F648 decays in 110 fs. As mentioned for the decay lifetime of the 560-nm fluorescence (see Sec. 3.3.4), Cars in this strain should energetically bound to DV-Chls. The second (800 fs–1.0 ps) and third (6.0–10.5 ps) lifetimes correspond to energy transfers among DV-Chl *a*, DV-Chl *b* and S_1 -state of Cars, as revealed by the FDA spectra. The second lifetimes are slightly longer than those in CCMP1986 (650–900 fs), indicating slightly weaker coupling between DV-Chl *b* and DV-Chl *a*. This difference becomes clearer in

the third lifetime components, where the lifetimes of DV-Chl *b* bands (F648 and F660) are 9.9 and 10.5 ps in CCMP1375, and 6.5 and 4.6 ps respectively in CCMP1986. On the other hand, the third lifetimes in the DV-Chl *a* fluorescence region do not appreciably differ between CCMP1375 and CCMP1986. The relative amplitude of F687 at time zero is slightly larger in CCMP1375 than in CCMP1986 (Fig. 3-4; also see Fig. 3-2), indicating that CCMP1375 contains more DV-Chl *a* (F687), possibly to enhance its light harvesting ability using the F687 component.

In the first lifetimes (100–140 fs) of CCMP2773, the amplitudes of all spectral components are negative. Fast decay of free Car S₂ is absent in the shorter wavelength region, because CCMP2773 contains sufficient DV-Chl *b* to hide the contribution of Cars. The second lifetimes (610 fs–1.0 ps) in this strain are shorter than those observed in CCMP1375 (except for F687), indicating more efficient energy transfer between the pigments. Comparing the third lifetime components of F648 and F660 in CCMP1375 and CCMP2773, both components decay faster in the latter strain (lifetime = 5.0 ps), indicating that DV-Chl *b* strongly couples with DV-Chl *a* in this strain. The amplitude around 0 ps in the F687 component is higher for CCMP2773 than

for the other two strains (Fig. 3-4), and the second lifetime (1.0 ps) is longer. Again, these trends are attributable to the DV-Chl *a* (F687) content. In addition, the decaying third components of F672 and F687 are unique to CCMP2773. This means that energy is transferred among the DV-Chl *a* molecules, namely, from F672 and F687 to F682. Judging from the amplitudes of the second and third lifetime components, the most important component in CCMP2773 is F687, which mediates energy transfer to F682. As indicated from the second FDA spectrum (830 fs, Fig. 3-3), DV-Chl *a* (F687) probably accepts excitation energy from the S_1 states of the Cars (especially α -carotene); thus, it acts as the primary mediator of energy transfer from the Cars to DV-Chl *a* (F682). The energy transfer efficiency from the S_1 state of α -carotene to F687, estimated from the second time constant of F687 (1.0 ps, Table 3-1) and the free decay lifetime of α -carotene S_1 (14.3 ps),⁸³ is as high as 93%. The third time constants of F672, F682, and F687 (4.6–5.0 ps) in CCMP2773 are comparable with or shorter than those obtained in CCMP1986 (5.6–6.0 ps) and CCMP1375 (6.0–8.3 ps). This indicates that *Prochlorococcus* can maintain excitation-energy-transfer to the reaction center while broadening the usable wavelength region by changing its pigment composition.

As a summary, the characteristic energy transfer pathways elucidated by the present study are shown in Fig. 3-5. The energy transfer pathways between DV-Chl *a* were not clearly resolved, except for that from F687 to F682. However, the energy transfers between DV-Chl *a* should occur to achieve excitation equilibrium within Chl-pigment pools with short lifetimes, because the spectral structure did not change in the ps–ns region.⁵⁷ Actually, it was reported that energy transfer between Chl *a* occurred in a few ps.^{5,6}

3.4.2 Comparison of pigment compositions among different light-harvesting systems

In chapter 3, the light harvesting functions in three *Prochlorococcus* strains with different pigment compositions were discussed. The structure of the absorption spectra around 500 nm depended on the light condition, indicating that *Prochlorococcus* can adapt to light conditions in the deep ocean by adjusting its pigment composition. Both DV-Chls and Cars play important roles in light harvesting by *Prochlorococcus*, but the light conditions in the organism's habitat largely determine the interaction between pigments.

The light-harvesting mechanisms in photosynthetic organisms are diversified by the pigment compositions or the light-harvesting complexes of the organisms. As is well known, many photosynthetic organisms contain Chl *b* and Chl *c*, which transfer excitation energy to Chl *a* with high efficiency. The absorption peaks of the Chls depend on the Chl conjugation structure; only (DV-)Chl *b* and Chl *c* absorb light with longer wavelengths in the Soret bands and shorter wavelengths in the Q_y bands than Chl *a*. In the green alga *Codium fragile* and the higher plant *Arabidopsis thaliana*, the lifetime of energy transfer from Chl *b* to Chl *a* is 700–800 fs, indicating an efficiency close to unity.^{5,6} Energy transfer between Chl *c* and Chl *a* is also efficient; lifetimes of 500–700 fs and 4–6 ps have been reported for intra-complex and inter-complex transfers, respectively, in the diatom *Chaetoceros gracilis*,^{7,9,10} and 1.4 ps in the dinoflagellate, *Amphidinium*.⁸⁴ According to these findings, (DV-)Chl *b* and Chl *c* are the efficient energy donors to (DV-)Chl *a*. Among these Chls, the wavelengths covered by the Soret band are longest in DV-Chl *b* (~480 nm; see Fig. 3-1). From a biological perspective, the ocean depths inhabited by *Prochlorococcus* narrow the sunlight spectrum to the blue–green region, so DV-Chl *b* should be the best pigment to collect light under these

light conditions.

Although the light-harvesting mechanisms in *Prochlorococcus* appear to be mainly mediated by the DV-Chls, energy transfer from Cars to DV-Chl *a* also plays an important role. All three of the investigated *Prochlorococcus* strains exhibited efficient energy transfer pathways from Cars to higher-energy DV-Chl *a*, via lower-energy DV-Chl *a* (corresponding to F687). The Cars in *Prochlorococcus* are α -carotene and zeaxanthin, which are conjugated polyenes containing only C=C bonds.^{85,86} A similar energy transfer process has been reported in *C. gracilis*; namely, energy is transferred from fucoxanthin, which involves a keto-carbonyl group in the conjugated double bond system, to higher-energy Chl *a* via lower-energy Chl *a* (with a fluorescence peak at 688 nm).^{10,87} Therefore, both polyene-type Cars and keto-Cars transfer excitation energy to lower-energy (DV-)Chls, which is then transferred to higher-energy (DV-)Chls.

The ultrafast energy transfer processes involving DV-Chls and Cars in the picocyanobacterium *Prochlorococcus marinus* were investigated in this study. The pigments in three strains of *Prochlorococcus* differed in their relative amount ratios and coupling conditions, depending on the light conditions of the cell's habitat or culture

environment. The lower-light adapted strain (CCMP1375 and CCMP2773) contained more DV-Chl *b* than its higher-light adapted counterparts (CCMP1986), but the decay time constants in all strains were of sub-ps order, implying super-efficient (almost unity) energy transfer from DV-Chl *b* to DV-Chl *a*. In the lower-light adapted strain, Cars function as important antenna pigments that transfer excitation energy to DV-Chl *a*. A characteristic energy pathway from Cars to higher-energy DV-Chl *a* band via lower-energy DV-Chl *a* band was also identified. By this mechanism, the lower-light adapted strain can broaden its usable range of light wavelengths.

3.5 Publication statement

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Figures and Table

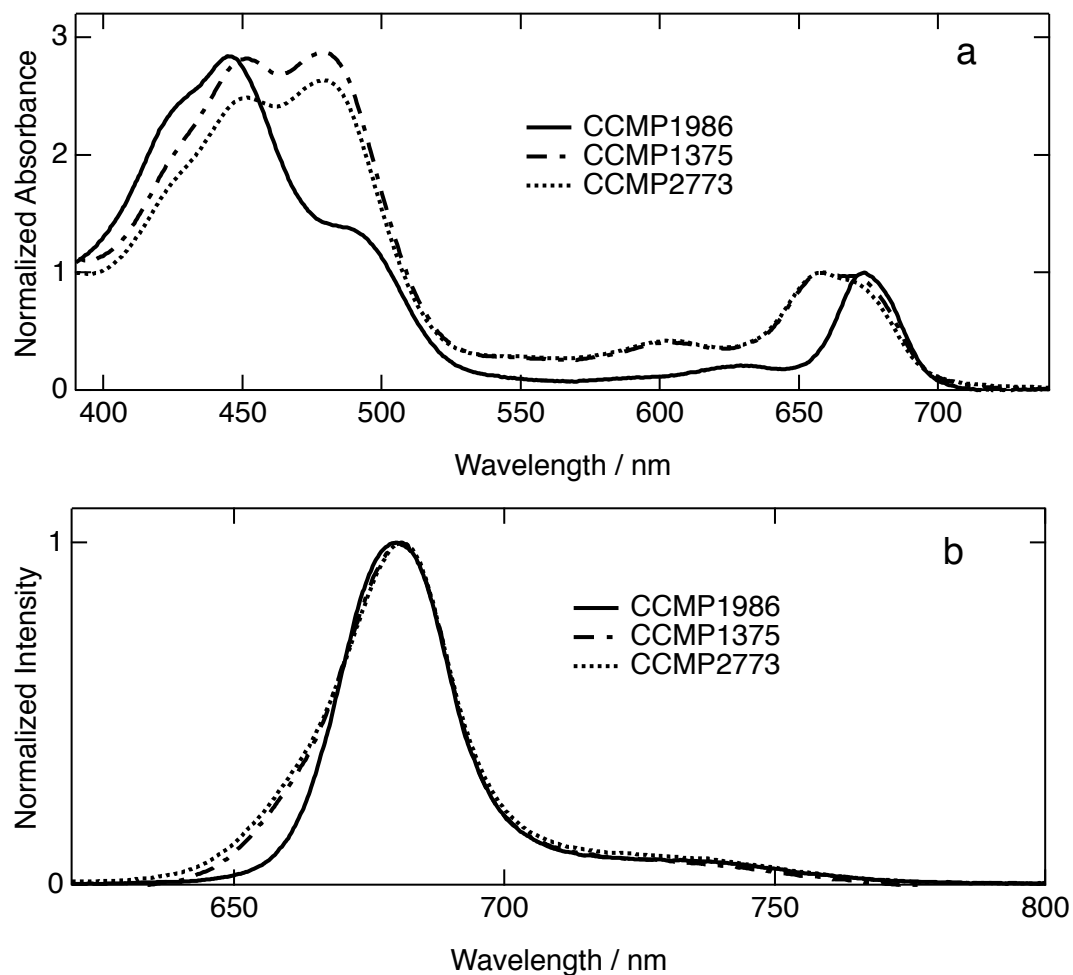


Figure 3-1. Steady-state spectra of *Prochlorococcus* CCMP1986 (solid line), CCMP1375 (dash-dotted line) and *Prochlorococcus* CCMP2773 (dotted line) at 293 K: (a) absorption spectra, normalized by the DV-Chl *a* Q_y band (CCMP1986) or the DV-Chl *b* Q_y band (CCMP1375 and CCMP2773); (b) fluorescence spectra excited at 425 nm, normalized by the peak wavelengths.

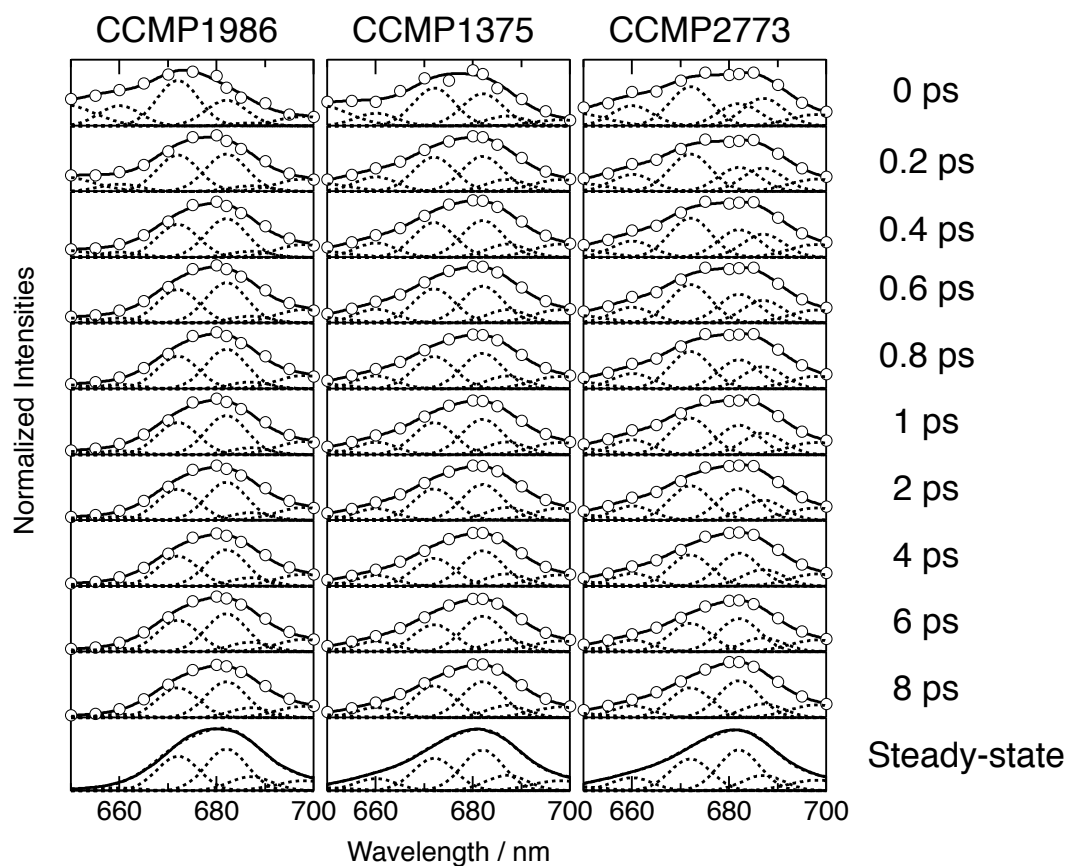


Figure 3-2. Time-resolved fluorescence (TRF) spectra of *Prochlorococcus* CCMP1986, CCMP1375, and CCMP2773 at 293 K. Open circles denote the measured data points, and the solid and dotted lines are the fitted curves and resolved spectral components, respectively. In the bottom windows (Steady-state), the solid lines show the steady-state spectra, and the dotted lines are the fitted curves and resolved spectral components.

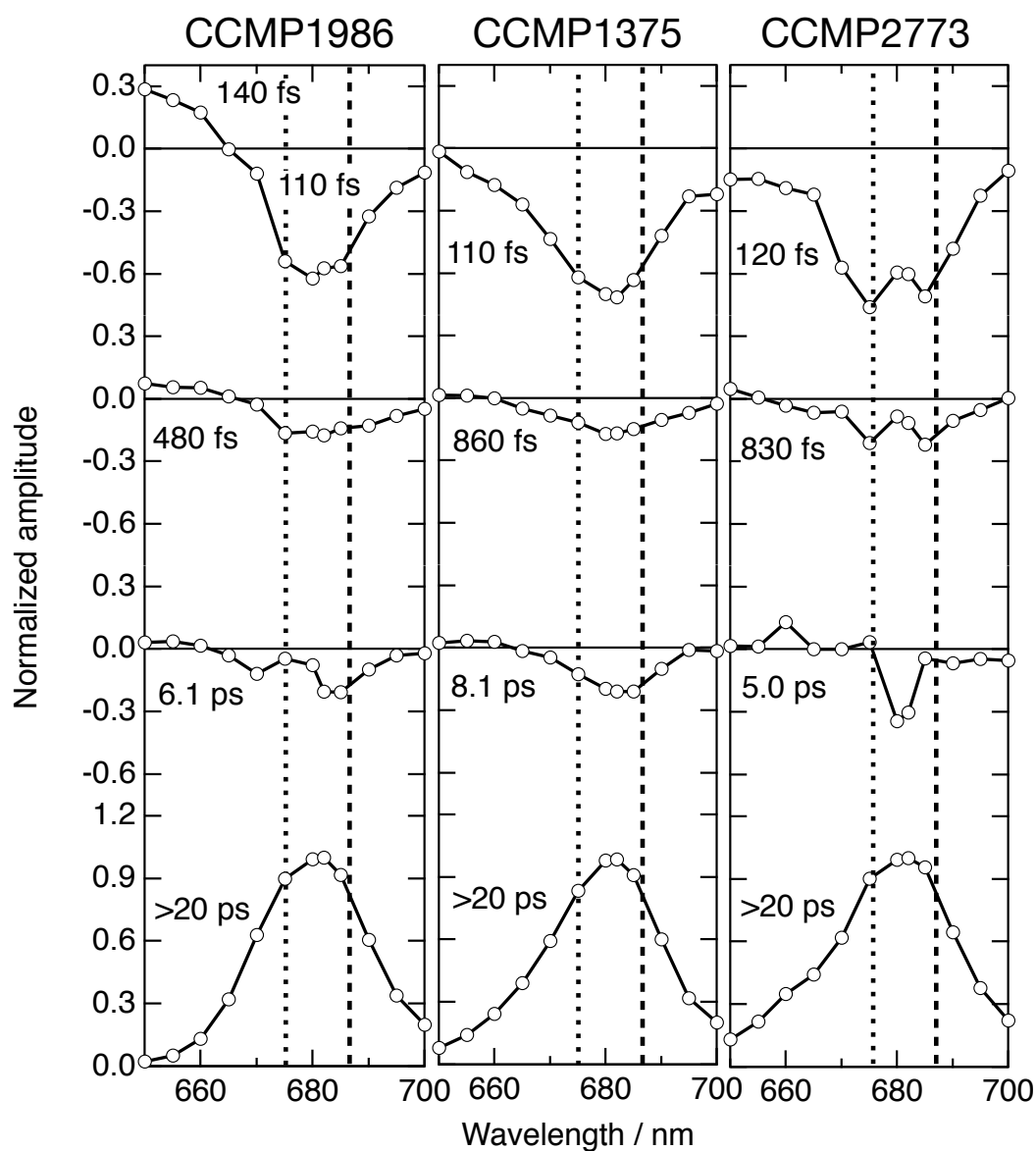


Figure 3-3. Fluorescence decay-associated (FDA) spectra for *Prochlorococcus* CCMP1986, CCMP1375, and CCMP2773, obtained from the global analysis. Vertical dotted lines (left) and broken lines (right) indicate the energy levels of the lowest electronically excited state in α -carotene and zeaxanthin, respectively.

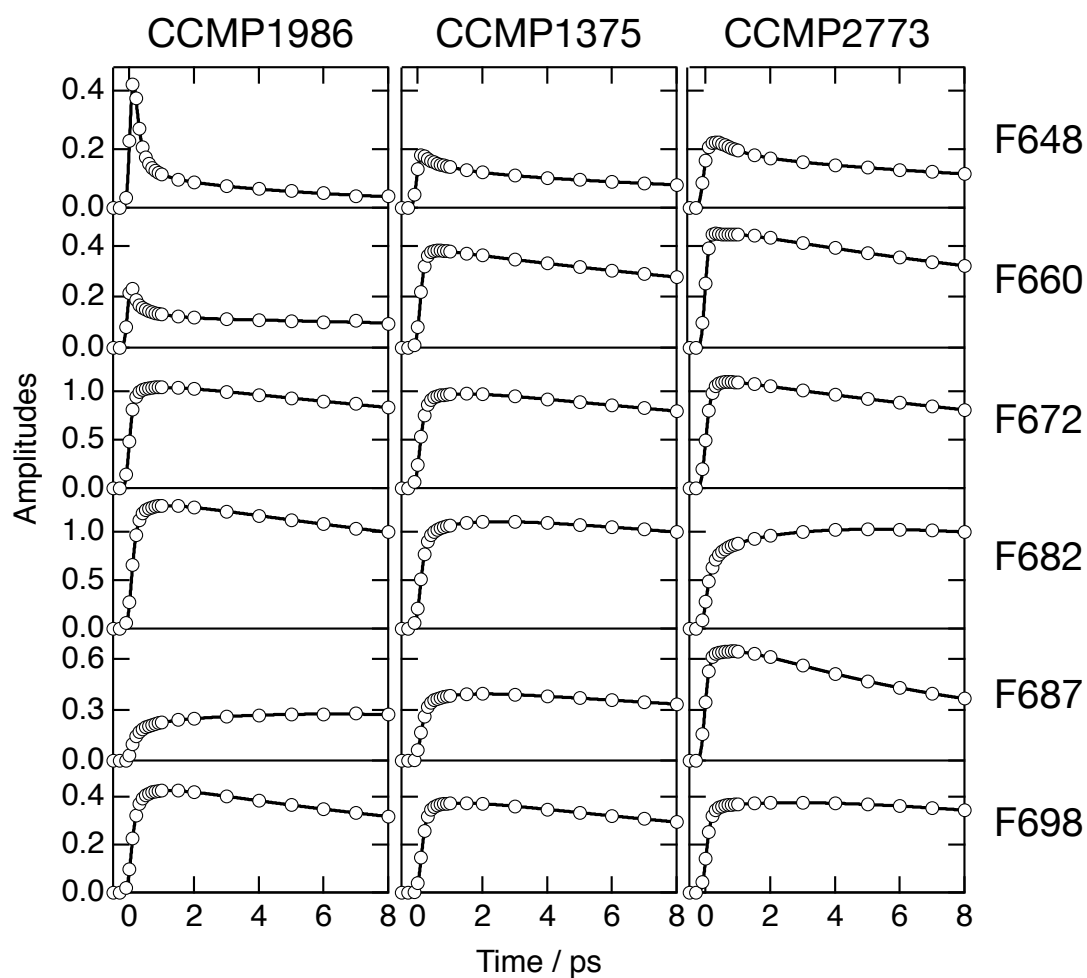


Figure 3-4. Time evolutions of the resolved spectral components shown in Fig. 3-2 (see Section 3.3.5 for description). All amplitudes are normalized by the amplitudes of the F682 components at 8 ps.

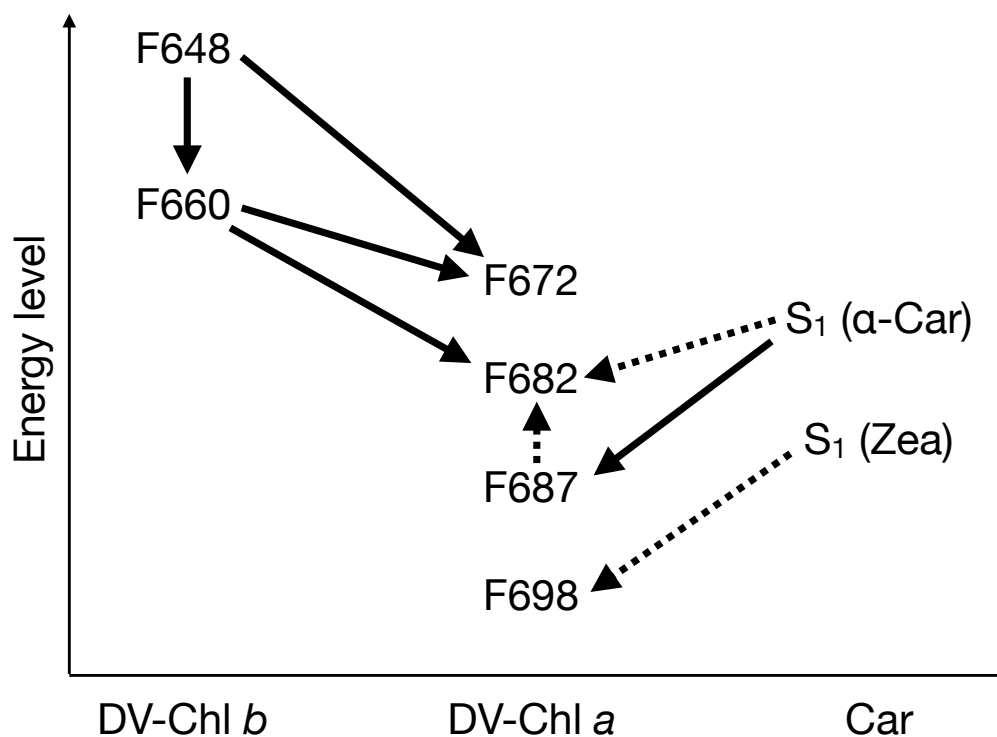


Figure 3-5. Energy-transfer pathways in *Prochlorococcus*. The solid arrows are the processes occurred in sub-ps, while the dotted arrows are those in 5–8 ps (see Sec. 3.4.1 for details). Solid lines are observed in all the three strains; dotted lines from Cars to DV-Chl *a* are in strains CCMP1375 and CCMP2773; and a dotted line from F687 to F682, in only CCMP2773.

Table 3-1. Lifetimes of the spectral components shown in Fig. 3-2, and their amplitudes

(normalized such that the positive amplitudes of each spectral component sum to unity).

Strains	Components	Lifetimes (Amplitudes)			
CCMP1986	F648	160 fs (0.77)	680 fs (0.09)	6.5 ps (0.12)	>20 ps (0.02)
	F660	130 fs (0.51)	900 fs (0.14)	4.6 ps (-0.08)	>20 ps (0.35)
	F672	100 fs (-0.45)	750 fs (-0.08)	6.0 ps (-0.10)	>20 ps (1.00)
	F682	120 fs (-0.71)	650 fs (-0.13)	6.2 ps (-0.05)	>20 ps (1.00)
	F687	110 fs (-0.13)	380 fs (-0.16)	5.6 ps (-0.56)	>20 ps (1.00)
	F698	130 fs (-0.62)	670 fs (-0.09)	4.7 ps (0.06)	>20 ps (0.94)
CCMP1375	F648	110 fs (0.25)	940 fs (0.20)	9.9 ps (0.24)	>20 ps (0.32)
	F660	120 fs (-0.44)	1.0 ps (-0.01)	10.5 ps (0.04)	>20 ps (0.95)
	F672	120 fs (-0.60)	990 fs (-0.12)	8.3 ps (-0.12)	>20 ps (1.00)
	F682	110 fs (-0.56)	800 fs (-0.13)	6.7 ps (-0.30)	>20 ps (1.00)
	F687	110 fs (-0.60)	860 fs (-0.20)	6.0 ps (-0.15)	>20 ps (1.00)
	F698	120 fs (-0.45)	990 fs (-0.14)	6.7 ps (-0.01)	>20 ps (1.00)
CCMP2773	F648	120 fs (-0.09)	730 fs (0.31)	5.0 ps (0.14)	>20 ps (0.55)
	F660	110 fs (-0.03)	1.0 ps (-0.07)	5.0 ps (0.09)	>20 ps (0.91)
	F672	130 fs (-0.97)	660 fs (-0.03)	4.6 ps (0.01)	>20 ps (0.99)
	F682	140 fs (-0.40)	610 fs (-0.10)	4.8 ps (-0.49)	>20 ps (1.00)
	F687	100 fs (-0.51)	1.0 ps (-0.21)	5.0 ps (0.49)	>20 ps (0.52)
	F698	110 fs (-0.47)	980 fs (-0.01)	5.0 ps (-0.32)	>20 ps (1.00)

Chapter 4. Adaptation of divinyl chlorophyll *a/b*-containing cyanobacterium

Prochlorococcus to different light conditions

4.1. Introduction

The natural habitat of DV-Chl *b*-rich *Prochlorococcus* is located at depths with only limited blue light.⁶⁰ Partensky et al. investigated the effect of blue light, which peaks at 475 nm with a full-width half-maximum (FWHM) of 100 nm, on DV-Chl *b*-rich CCMP1375 and DV-Chl *a*-rich CCMP1986.⁶¹ They showed that the content ratio of DV-Chl *b*/DV-Chl *a* in CCMP1375 decreased compared with that for white-light growth for the corresponding intensity. In the present work, the effects of a blue LED with sharper FWHM, which is absorbed by DV-Chl *b*, as well as those of a red LED, absorbed by DV-Chl *a*, on the light-harvesting function of *Prochlorococcus* are investigated. The excitation-energy-transfer process and pigment contents of *Prochlorococcus* grown under different light colors and intensities are compared using ps TRF measurements and absorption spectra, respectively.

4.2. Materials and methods

4.2.1 Cell growth conditions

Three strains of *Prochlorococcus*, CCMP1986, CCMP1375, and CCMP2773, were grown autotrophically in PCR-S11 medium at 293 K (20 °C).⁵⁷ First, the *Prochlorococcus* cells were grown under a white fluorescent lamp in a 12-h light–12-h dark regime, and the light intensities were adjusted according to the strains: 40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for the DV-Chl *a*-rich strain (CCMP1986) and 4 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for the DV-Chl *b*-rich strains (CCMP1375 and CCMP2773), as reported elsewhere.⁵⁷ After the growth under the fluorescent lamp, the cells were grown under blue or red LEDs using the same time regimes. Spectral profiles of the blue and red LEDs are presented in Fig. 4-1 together with typical absorption spectra of *Prochlorococcus*. The blue LED light peaks at 475 nm with a 26-nm FWHM (blue line in Fig. 4-1), which selectively excites DV-Chl *b*, whereas the filtered red LED light peaks at 685 nm with a 23-nm FWHM (red line in Fig. 4-1), which selectively excites DV-Chl *a*. The LED light intensities were set at two levels for each strain: 40 or 20 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for CCMP1986 and 4 or 2 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for CCMP1375 and CCMP2773. The cells

were grown under the LED light for several weeks before the measurements. Only CCMP1375 exhibited remarkable changes in its absorption spectra depending on the light color (see sec. 4.3.1). Therefore, the recovery in the absorption spectra after light color alternation was examined; the CCMP1375 grown under the red LED with an intensity of $4 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ was transplanted to two different light conditions: $4 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ of blue LED light and $4 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ of the red LED light. The cells were grown under the different light conditions for several weeks before the measurements.

4.2.2 Absorption and fluorescence spectroscopy measurements

Steady-state absorption and fluorescence spectra were measured using a spectrometer (V-650, JASCO, Japan) and fluorometer (FP-6600 with ILFC-543L, JASCO, Japan), respectively. TRF was measured using the time-correlated single photon-counting method with an excitation wavelength 408 nm, as reported elsewhere.⁸⁸ The samples were mixed with the same volume of poly (ethylene glycol) 4000 (30 %, w/v) (Wako Pure Chemicals, Japan) to obtain homogeneous ice and were

frozen in the dark. The time interval for the data acquisition was set to 2.4 or 24.4 ps/channel. The repetition rate of the pulse was 5 MHz, which does not affect the timeframe of 100-ns measurements by 4096 channels. The steady-state fluorescence and TRF spectra were measured at 77 K. The fluorescence lifetime was estimated using a convolution calculation.⁸⁹ FDA spectra were constructed by global analysis (see sec. 2.3.4).³³ The measured fluorescence rise and decay curves were analyzed by the convolution of the exponentials (Eq. 1 in sec. 2.3.4) and the instrumental response function measured by scattering from the sample. The resulting FDA spectra reveal the excitation-energy-transfer pathways. In each FDA spectrum, negative and positive regions are observed, which correspond to fluorescence rise and decay signals, respectively. Coupling of positive and negative bands indicates the energy transfer from a pigment with a positive band (donor) to that with a negative band (acceptor).

4.2.3 Pigment extraction and identification

Pigments in the *Prochlorococcus* cells were extracted in a microfuge tube with acetone. After mixing by a stainless spatula, the tube was centrifuged at 10000 rpm

for 5 min with cooling. The absorption spectra of the supernatant at 298 K was measured for the identification analysis. The spectra were analyzed with the components corresponding to the absorption spectra of DV-Chl *a*, DV-Chl *b*, α -carotene, and zeaxanthin. The spectra and the result of analysis are shown in Fig. 4-10. The pigment amount ratio derived by the analysis is summarized in Table 4-1.

4.3. Results

4.3.1 Steady-state absorption spectra

Fig. 4-2 shows the steady-state absorption spectra of the blue- and red-LED-grown cells of *Prochlorococcus* at 298 K. Hereafter, the light conditions with the higher intensities (40 or $4 \mu\text{mol photons m}^{-2}\text{s}^{-1}$) of the blue or red LED will be referred to as B40 (blue LED, $40 \mu\text{mol photons m}^{-2}\text{s}^{-1}$), R40 (red LED, $40 \mu\text{mol photons m}^{-2}\text{s}^{-1}$), B4 (blue LED, $4 \mu\text{mol photons m}^{-2}\text{s}^{-1}$), or R4 (red LED, $4 \mu\text{mol photons m}^{-2}\text{s}^{-1}$), and those with lower intensities will be referred to as B20, R20, B2, and R2. The bands at 445 and 673 nm with vibrational bands at approximately 430 and 620 nm, respectively, which are apparently observed in the spectra of the CCMP1986

cells, are attributed to DV-Chl *a*.^{60,72} The $S_2 \leftarrow S_0$ absorption bands of the Cars (α -carotene and zeaxanthin) appear at approximately 480–490 nm.⁴ The bands at 480 and 658 nm, which are clearly observed in the spectra of CCMP1375 and CCMP2773 cells grown under the white light, are assigned as DV-Chl *b* bands.^{60,72} The spectral contours of the blue-LED-grown cells do not differ significantly from those of the white-light-grown cells.⁵⁹ In the red-LED-grown cells of CCMP1375 and CCMP2773, the relative absorbance of DV-Chl *b* is reduced compared to that of the blue-LED-grown cells. In the spectrum of the red-LED-grown CCMP1375 cells, the intensity of the Soret band of DV-Chl *b* decreases, and another band appears on the shorter-wavelength side. This behavior was also reported for CCMP1375 grown under strong white light.⁶¹ In all the strains, the absorbance for wavelengths shorter than 500 nm is largest for the red-LED-grown cells, especially for the higher intensities, R40 for CCMP1986 and R4 for CCMP1375 and CCMP2773.

Fig. 4-3 shows the absorption spectra of CCMP1375 cells transplanted from the R4 condition to B4 or R4 conditions (referred to as RB4- or RR4-grown cells, respectively). The spectral contour of the RB4-grown cells is almost identical to that of

the B4-grown cells (Fig. 4-2), indicating that the CCMP1375 cells adapted to the blue LED again after the spectral change in the Chl Soret band under the R4 light condition. The spectral contour of the RR4-grown cells is close to that of the R4-grown cells (Fig. 4-2), except for the small difference of the relative intensity in the Chl Soret band or Car band region.

4.3.2 Steady-state fluorescence spectra

Fig. 4-4 shows the normalized steady-state fluorescence emission spectra of the three strains of *Prochlorococcus* (except the RB4- and RR4-grown cells of CCMP1375) at 77 K. The excitation wavelength was 445, 475, or 500 nm, which excites DV-Chl *a*, DV-Chl *b*, or Cars, respectively. Some differences are observed for wavelengths longer than 695 nm between the cells grown under different light colors/intensities. For CCMP1986, the fluorescence around 700–730 nm, which can be attributed to the signal of PSI, is more intense in the red-LED-grown cells than in the blue-LED-grown ones, independent of the excitation wavelength.^{29,71,90} In addition, for

growth under the same color LED, the PSI fluorescence is more intense for the cells grown under lower intensities. For CCMP1375, a different tendency is observed; the PSI fluorescence is more intense for the blue-LED-grown cells than for the red-LED-grown ones, especially by the 500-nm excitation. However, for growth under the same color LED light, the B4- and R4-grown cells showed higher intensities than the B2- and R2-grown cells, with an even clearer difference between the B4- and B2-grown cells. For CCMP2773, the difference by the light conditions is smaller than that for CCMP1986 and CCMP1375. However, a difference in the shorter-wavelength region at approximately 670 nm is observed in CCMP2773, especially by the 500-nm excitation; the blue-LED-grown cells showed higher-intensity in this region, whereas no clear differences were observed between the different LED intensities. In the PSI fluorescence region, the blue-LED-grown cells exhibited slightly higher-intensity peaks than the red-LED-grown cells. The difference between the LED intensities is not clear in the PSI fluorescence region.

Fig. 4-5 shows the steady-state fluorescence emission spectra of the RB4- and RR4-grown cells of CCMP1375. As with the absorption spectra, the steady-state

fluorescence spectra of the RB4-grown cells are very close to those of the B4-grown cells (Fig. 4-4). However, those of the RR4-grown cells differ from those of the R4-grown cells (Fig. 4-4) in some points. In the 660–680 nm region, which corresponds to the shorter-wavelength region of the DV-Chl *a* signal, the RR4-grown cells exhibit relatively intense signals compared with the R4-grown cells. In the PSI fluorescence region, the RR4-grown cells exhibit reduced signals, especially for the 445-nm excitation.

4.3.3 Time-resolved fluorescence spectra

Fig. 4-6 shows the TRF spectra of *Prochlorococcus* (except for the RB4- and RR4-grown cells of CCMP1375) at 77 K. For all the cells, the spectral band shape becomes sharper in the latter times, except for the PSI fluorescence region at approximately 695–730 nm for timeframes of 4.5–5.7 ns. In the latter timeframes of 15–17 ns and 39–46 ns, the PSI fluorescence is not clearly detected. For the blue-LED-grown cells of CCMP1986, the fluorescence intensity peak is observed at

684 nm in the 0–5-ps timeframe and it shifts to 688 nm after 210 ps. The same tendency is observed for the red-LED-grown cells of CCMP1986. The seeable difference between CCMP1986 grown under the blue and red LED is the relative intensity of the signals in the 670–680 nm region, especially for the timeframes of 0–5 ps and 4.5–5.7 ns, and in the 695–730 nm region, especially for the 4.5–5.7 ns timeframes. When comparing the LED intensities, the B40- and R40-grown cells exhibited higher signal intensities in those regions (670–680 and 695–730 nm) than the B20- and R20-grown cells. In addition, the different characteristics of the TRF spectra between the different LED colors and intensities are clearer for CCMP1375 than for CCMP1986. Clear differences in the spectral contours are also apparent for the different LED colors and intensities. The B4-grown cells exhibited relatively higher intensities at wavelengths longer than 695 nm in all the timeframes, especially 85–110 ps, 0.9–1.1 ns, and 4.5–5.7 ns. The B4- and B2-grown cells exhibited relatively higher intensities compared with those of the R4- and R2-grown cells for 670–680 nm, especially in the 85–110-ps timeframe. For CCMP2773, the signal differences between the cells grown under different LED colors and intensities are smaller than for CCMP1375. The fluorescence intensity peaks in the

0–5-ps timeframe were located at 687 nm for the cells grown under all of the light conditions. Differences between the cells grown under the different LED colors and intensities are observed in the 695–730 nm region for 4.5–5.7 ns and in the 670–680 nm region for 0–5 ps. In both cases, the order of the relative intensity is B4- > B2- > R4- > R2-grown cells.

Fig. 4-7 presents TRF spectra of the RB4- and RR4-grown cells of CCMP1375. The differences in the spectral contours between the two cells are comparable to those between the B4- and R4-grown cells. When the B4- and RB4-grown cells (Figs. 4-6 and 4-7, respectively) are compared, few differences are observed, except for 670–680 nm region in the first 0–5 ps and second 85–110 ps timeframes. The same type of difference is observed between the R4- and RR4-grown cells, with the RR4-grown cells exhibiting higher intensity in the 670–680 nm region at 0–5 ps and 85–110 ps. The relative intensities for wavelengths longer than 695 nm was clearly different between the R4- and RR4-grown cells in the steady-state spectra; however, in the TRF spectra, the difference was recognized only in the latter timeframes after 4.5–5.7 ns and was not so significant.

4.3.4 Fluorescence decay-associated spectra

FDA spectra (Figs. 4-8 and 4-9) were constructed by global analysis (see Sec. 2.3.4). The FDA spectra of *Prochlorococcus* grown under the LEDs were composed of 5 or 6 components: 10–40 ps, 170–280 ps, 500–920 ps, 1.4–4.3 ns, 5.5–8.1 ns, and 16–36 ns. For some cells, the 5th component, 5.5–8.1 ns, was not necessarily required for fitting. Fig. 4-8 shows the FDA spectra of the cells of the three strains grown under the blue or red LED except for the RB4- and RR4-grown cells of CCMP1375. The FDA spectra were normalized at the maximum amplitude of the second component (170–280 ps). The FDA spectra with the longest time constants, 16–36 ns, are attributed to the delayed fluorescence originating from the charge recombination occurring in the PSII reaction center.⁶⁴ The delayed fluorescence shows peaks in the PSII fluorescence region, and no peaks are observed in the PSI fluorescence region in any of the cells, indicating that in all *Prochlorococcus* cells, only PSI and PSII complexes exist and a PSI–PSII supercomplex is not formed. This behavior differs from that of other Chl *b*-containing cyanobacteria, *Prochloron* and *Prochlorothrix*, in which the PSII–PSI complexes are present. For *Prochlorococcus* CCMP1986, the time constants and spectral shapes of the

FDA spectra are comparable between the cells grown under different light conditions. The first components with time constants of 10 ps indicate the energy transfer mainly between DV-Chl *a*, whereas DV-Chl *b* only marginally contributes to the spectra because the amount of DV-Chl *b* in CCMP1986 is very small.^{57,59} The second to fourth components represent the slower energy-transfer processes between DV-Chl *a*, and the last components with long lifetimes of 16–29 ns represent delayed fluorescence. For CCMP1375, the number of components and time constants differ between the cells grown under the different light conditions. The time constants of the first FDA spectra are 40, 25, 40, and 15 ps for the B2-, B4-, R2-, and R4-grown cells, respectively. The lifetimes of the second to sixth FDA spectra are shorter for the blue-LED-grown cells. In addition, the lifetimes of the second to sixth FDA spectra are shorter for the B2- and R2-grown cells than for the B4- and R4-grown cells. The similar tendencies are observed for CCMP2773. The lifetimes of the third to fifth FDA spectra are shorter for the blue-LED-grown cells. In addition, the lifetimes of the second to sixth FDA spectra are shorter for the B2- and R2-grown cells than for the B4- and R4-grown cells. The clear difference from the FDA spectra of CCMP1375 is that the time constants of the

first FDA spectra of CCMP2773 cells are comparable to each other.

Fig. 4-9 shows the FDA spectra of the RB4- and RR4-grown cells of CCMP1375. The FDA spectra are normalized to the maximum amplitude of the second spectrum (230 ps). Some differences are observed between the B4-grown cells (Fig. 4-8) and RB4-grown cells (Fig. 4-9) that were not apparently recognized in the steady-state fluorescence and TRF spectra (Figs. 4-4, 4-5, 4-6, 4-7). The time constants and spectral contours of the first FDA spectra differ vastly. A significant decay signal for wavelengths shorter than 684 nm for the first FDA spectrum of the RB4-grown cells is observed, as with the relatively intense signal near this wavelength region in the TRF spectra for the early timeframes of the RB4-grown cells. As with the time constant of the RB4-grown cells (15 ps) being shorter than that of the B4-grown cells (40 ps), this finding indicates the efficient energy transfer from DV-Chl *a* in the peripheral antenna to PSII or PSI.^{2,12} The FDA spectra of the R4- and RR4-grown cells differ in the number of components; however, the contribution of the fifth FDA spectrum of the R4-grown cells is small. The time constants and spectral shapes of the FDA spectra for time constants of less than 830 or 850 ps are comparable to each other.

Fig. 4-10 and Table 4-1 shows the analytical result of the absorption spectra of the cells of the three strains, grown under the intensities of 40 or 4 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. The R40-grown cells of CCMP1986 have more amount of the Cars (and DV-Chl *b*) than the B40-grown cells. The R4-grown cells of CCMP1375 and CCMP2773 have more amount of zeaxanthin and less amount of DV-Chl *b* and α -carotene than the B4-grown cells.

4.4. Discussion

4.4.1 Dependence of light-harvesting functions on light conditions

In the both photosystems, the excitation-transfer processes differ between the three strains and varying light conditions. Comparing the FDA spectra of CCMP1986 with those of CCMP1375 and CCMP2773, the relative amplitude of the third (560–670 ps) and fourth (1.9–2.9 ns) FDA spectra are larger for CCMP1986. The larger contributions of the FDA spectra with these time constants indicate that the excitation energy transfer between DV-Chl *a* within both PSII and PSI is less efficient in

CCMP1986. This result may arise from the natural habitat of CCMP1986, which is under the relatively higher light conditions of the sea compared with the other two strains. For all the three strains, the excitation-energy-transfer efficiency between DV-Chl *a* are not significantly changed under the different light colors and intensities judging from the time constants of the second to fifth or sixth FDA spectra (Fig. 4-8). The excitation-energy-transfer process from DV-Chl *b* to DV-Chl *a* is not included in the second to sixth FDA spectra because the signal of DV-Chl *b* in the shorter wavelength region is not recognized.

In the steady-state fluorescence spectra (Figs. 4-4 and 4-5) and TRF spectra (Figs. 4-6 and 4-7), the relative intensities in the region of PSI fluorescence (approximately 690–730 nm) differ depending on the light color and intensity. However, in the FDA spectra with the longest time constants (Figs. 4-8 and 4-9), no bands assigned to the PSI fluorescence are observed in any of the cells; therefore, the excitation energy transfer in the pathway from DV-Chl *a* in PSII to DV-Chl *a* in PSI is marginal.¹⁵ These findings indicate that the observed difference in the relative intensity at approximately 695–730 nm originates from the difference in the energetically binding

condition between the antenna pigments mainly in the peripheral antenna protein Pcb and lower energy DV-Chl *a* in the photosystems.^{45,62,71} The excitation energy in PSII and PSI appears to be balanced by the binding conditions between the antenna protein Pcb and each photosystem. The relative fluorescence intensities of PSI/PSII differ for the different light colors and intensities; however, a common trend is not observed (Fig. 4-4). In CCMP1986, the PSI signal is more intense when grown under the red LED, whereas in CCMP1375, it is more intense when grown under the blue LED. In CCMP2773, the difference in the PSI/PSII ratio is less clear than that for the other two strains.

Among the first FDA spectra of the four samples of CCMP1375 in Fig. 4-8, the R4-grown cells exhibit the shortest time constant of 15 ps, which is comparable to the fastest time constants for the other two strains (10–15 ps). The 10–15-ps energy transfer may be mainly between DV-Chl *a*. In contrast, in the B4, B2, and R2-grown cells of CCMP1375, the time constants are longer (25–40 ps). This finding indicates the possibility that fast-energy-transfer processes from DV-Chl *b* to DV-Chl *a* or between DV-Chl *a* in Pcb, which are undetectable at the time resolution of the system (≤ 5 ps),

are present in the shorter-wavelength region of these cells. In the previous work, the excitation energy transfer from DV-Chl *b* to DV-Chl *a* was observed with time constants of less than 1 ps.⁵⁹ As the result, the analyzed time constants of the first FDA spectra of the B4-, B2-, and R2-grown cells of CCMP1375 may be longer than that of the R4-grown cells of CCMP1375 (and those of the other two strains). As observed in Fig. 4-4, the Cars in the blue-LED-grown cells of CCMP1375 (especially the B4-grown cells) are more energetically bound to DV-Chl *a* in PSI because the differences among the relative intensities of PSI fluorescence in CCMP1375 are more evident for the various light conditions by the 500-nm excitation compared with the excitations by the shorter wavelength. The difference is less clear in the other two strains.

When the R4-grown cells of CCMP1375 were transplanted to the RB4 and RR4 light conditions (in Fig. 4-9), the time constants of the first to fourth FDA spectra did not change significantly (in Fig. 4-8), and the FDA spectra of the RB4- and RR4-grown cells were comparable to each other. It is likely that the slower energy-transfer processes between DV-Chl *a* in the photosystems are similar between the RB4- and RR4-grown cells. The main difference between the different light

conditions is the time constants of the first FDA spectra, which is because of the excitation-energy-transfer efficiency between Pcb and the photosystems. This finding indicates that CCMP1375 modifies the excitation-energy-transfer efficiency from antenna pigments in Pcb to DV-Chl *a* in the photosystems, depending on the light conditions, without affecting the excitation-energy-transfer process between DV-Chl *a* in the photosystems. In contrast, in all the FDA spectra of CCMP2773 (in Fig. 4-8), the time constants and spectral shapes are not different significantly between the cells under the different light conditions. It appears that CCMP2773 modifies the relative amount of Pcb. In addition, CCMP1375 and CCMP2773 use different mechanisms to adapt to the light conditions even though both are DV-Chl *b*-rich *Prochlorococcus*.

4.4.2 Modification of pigment amount depending on the light conditions

Some changes in the absorption spectra of *Prochlorococcus* are observed between different light conditions (Fig. 4-2). When comparing the absorption spectra of the red- and blue-LED-grown cells, the relative intensities of DV-Chl *a* and DV-Chl *b*

change in the Qy bands of CCMP1375 and CCMP2773, whereas no clear modifications in the shape are observed for CCMP1986. The absorption spectral shape of the cells grown under the blue LED light, which is comparable to the light conditions of the natural habitat of the two strains, CCMP1375 and CCMP2773, do not change significantly compared with the spectral shape of the cells grown under the white light. In contrast, for the cells grown under the red LED light, the absorption spectra of CCMP1375 and CCMP2773 are clearly different from those under the different light conditions. Compared with those of the blue-LED-grown cells, the absorption bands of DV-Chl *b* were clearly reduced both for the Soret bands and Qy bands in the red-LED-grown cells. In addition to DV-Chls, the relative content of Cars changed depending on the light conditions in the three strains. For CCMP1986, the absorbance in the wavelength region shorter than 500 nm is larger under the red LED (Fig. 4-2), suggesting that the relative content of Cars is larger under the red LED than under the blue LED. The analysis of the relative pigment amount ratio in the cells (Fig. 4-10 and Table 4-1) supports the discussion. In CCMP2773, the absorbance in the wavelength region shorter than 500 nm is also different between the light conditions (Fig. 4-2). Fig.

4-10 and Table 4-1 proves that the content of DV-Chl *b* in CCMP2773 cells is smaller under the red LED. The content of α -carotene is also smaller, while that of zeaxanthin is larger in the red LED-grown cells. In CCMP1375, the red-LED-grown cells show the specific change in the spectral shape around the Chl Soret band. The reason is not clear in Fig. 4-2, but Fig. 4-10 and Table 4-1 shows that the relative content of DV-Chl *b* is significantly smaller in the red LED-grown cells. The phenomenon that the content of DV-Chl *b* and α -carotene is reduced while that of zeaxanthin is increased is in the same tendency with CCMP2773, but the significant change in the DV-Chl *b* content affected the spectral shape of CCMP1375 cells. These phenomena, the change in the relative amount of DV-Chls and the peak shift in CCMP1375, are reported elsewhere under different intensities of white light. In the works by Partensky et al. and Moore et al., the modification of the relative Chl content in CCMP1375 as a function of the intensity of white light was investigated.^{61,63} Under higher light intensities, the amount of DV-Chl *b* decreased; furthermore, the amount of zeaxanthin was increased.⁶¹ In contrast, the present work represents the first time that phenomena like these are observed under different light colors with the same intensities. The possible reason why the amount of

zeaxanthin increased and those of DV-Chl *b* and α -carotene decreased under the red LED is that CCMP1375 changes the pigment composition by accumulating the different types of Pcb under the red LED.⁵⁴ It should be noted here that CCMP1375 has seven *pcb* genes, whereas CCMP1986 and CCMP2773 have one (*pcbA*) and two (*pcbA* and *pcbB*), respectively.⁵⁴ CCMP1986 modifies the amount of Cars depending on the light conditions, but the spectral shape in the wavelength region shorter than 500 nm is not changing apparently, possibly because CCMP1986 has only one type of Pcb (PcbA) and the amount ratio of the Cars in the PcbA is constant. The result that the amount ratio of DV-Chl *a/b* was not significantly different under the different light conditions in CCMP1986, supports the discussion that CCMP1986 modify the amount of only PcbA between the different light conditions. On the other hand, the DV-Chl *b*-rich *Prochlorococcus*, especially CCMP1375, which contains much more types of Pcb proteins, can adapt to the light color even when the light is of different wavelength component than that of its natural habitat. In addition, as observed in Fig. 4-3, CCMP1375 can adapt backward to the blue LED by altering the relative pigment amount. The profiles of the absorption spectra of the B4- and RB4-grown cells are

similar. It appears that CCMP1375 can alter the relative amount ratio of several Pcb types bound to the photosystems depending on the light conditions, and that CCMP2773 can also modify the relative amount ratio of the two types of Pcb, PcbA and PcbB.

In the present study, the relative pigment amount ratios in *Prochlorococcus* CCMP1986, CCMP1375 and CCMP2773 changed depending on the light color and intensity for the growth. In CCMP1375 and CCMP2773, red LED light induced less DV-Chl *b* content than blue LED light. In addition, in CCMP1375, the relative amount of zeaxanthin and α -carotene changed. These changes in the pigment amount ratio might be because of the change in the amount ratio of the Pcb types under the red LED light.

The light-harvesting function of *Prochlorococcus* grown under various light colors and intensities was investigated by the ps time-resolved fluorescence spectroscopy. For growth under red LED, which differs greatly from the light conditions of the natural habitat of *Prochlorococcus*, the properties of the absorption spectra of the DV-Chl *b*-rich strains, CCMP2773 and especially CCMP1375, changed significantly.

This result is due to the change in the relative amount ratio of the pigments. In addition, in CCMP1375, the pigment composition was remodified to the original under the blue LED light. However, the larger time constants of the energy transfer between DV-Chl *a* in the photosystems of CCMP1375 did not change depending on the light conditions, though a difference in the energy-transfer efficiency between Pcb and the photosystems was observed in the first FDA spectrum. DV-Chl *a*-rich CCMP1986 did not show any significant change in the properties of the absorption spectra or the energy-transfer time constants. It was found that among three strains examined here, the energy-transfer time constants are modified depending on the light conditions only in CCMP1375. The varieties of Pcb seems to affect energy transfer in CCMP1375.

4.5 Publication statement

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Strains of *Prochlorococcus marinus*, *J. Phys. Chem. B* **2017**, 121, 9081–9090.

Figures and Table

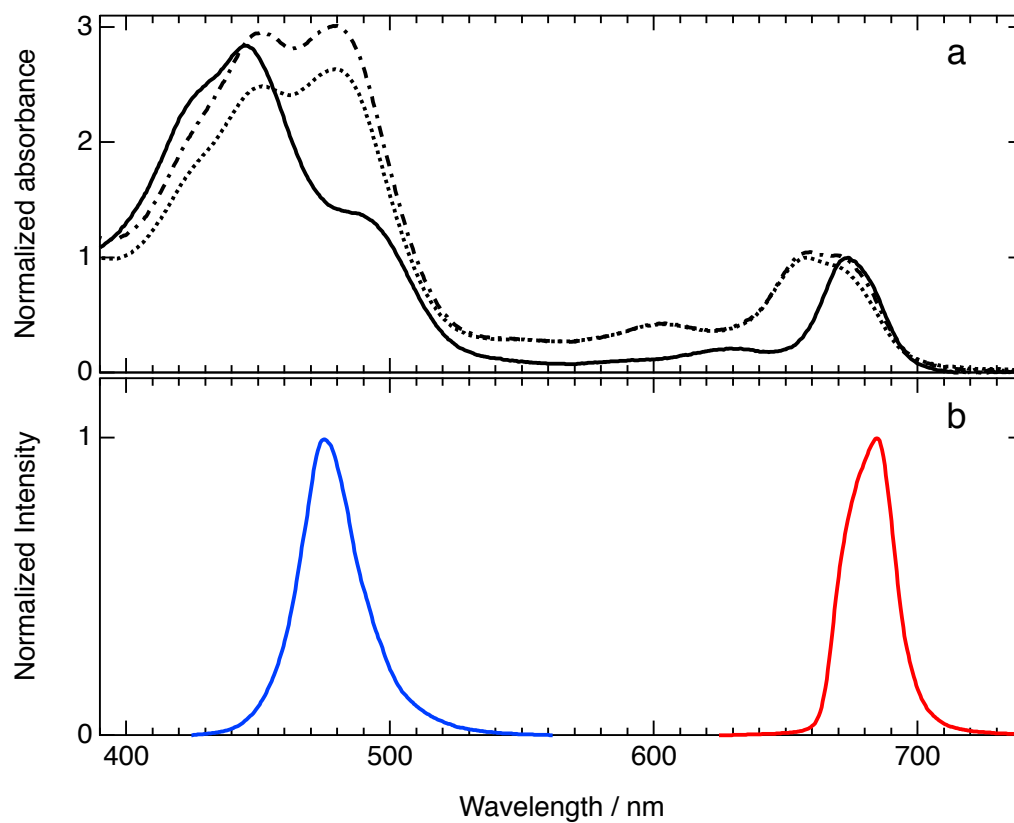


Figure 4-1. (a) Steady-state absorption spectra of the three *Prochlorococcus* strains, CCMP1986 (black solid line), CCMP1375 (black dash-dotted line), and CCMP2773 (black dotted line), grown under a fluorescent lamp. (b) Spectra of blue LED (blue line) and red LED (red line).

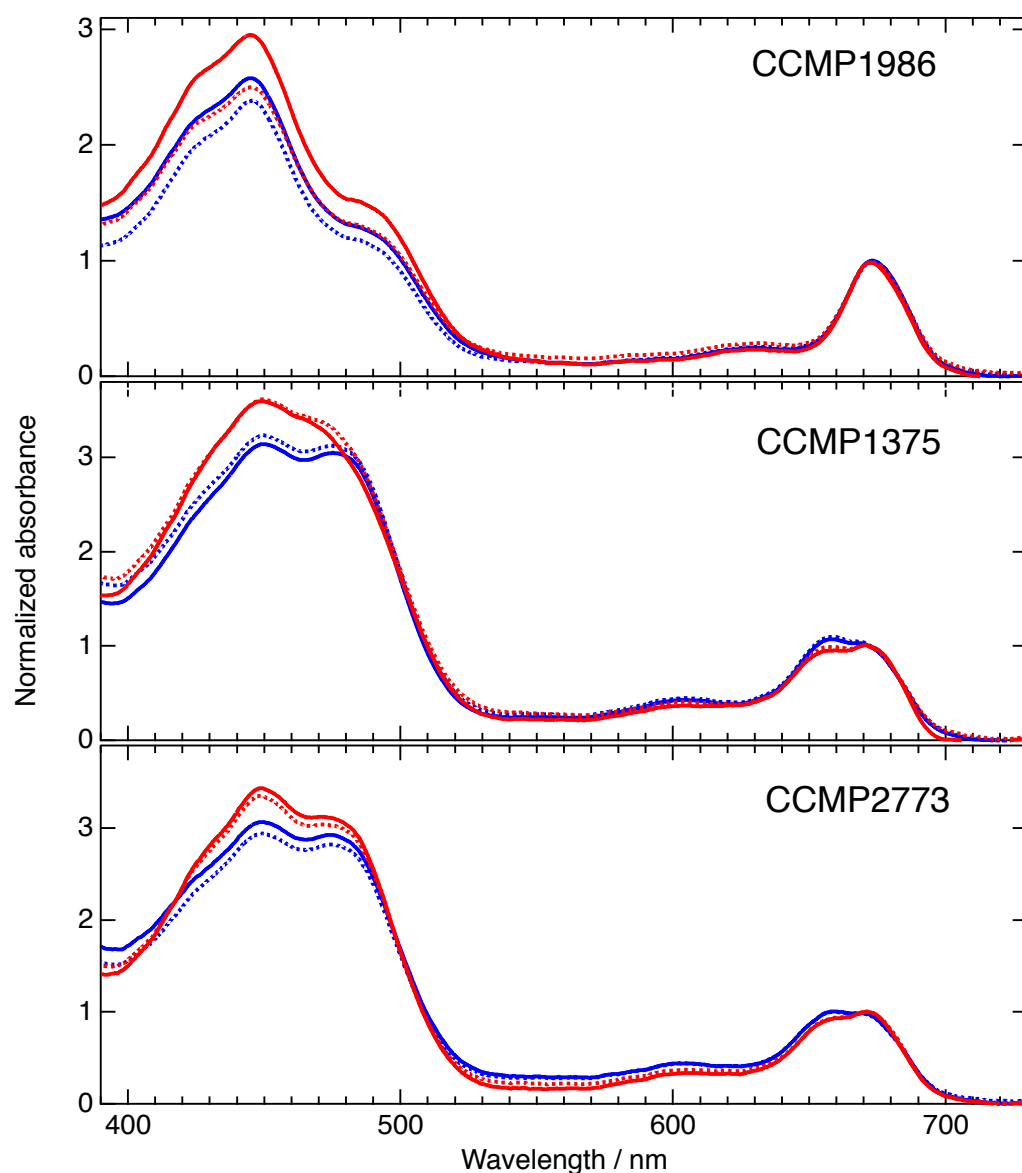


Figure 4-2. Steady-state absorption spectra of the three *Prochlorococcus* strains, CCMP1986 (top), CCMP1375 (middle), and CCMP2773 (bottom), grown under various light conditions. The blue and red lines represent the cells grown under blue and red LEDs, respectively. The solid and dotted lines represent the cells grown under LEDs with higher and lower intensities, respectively.

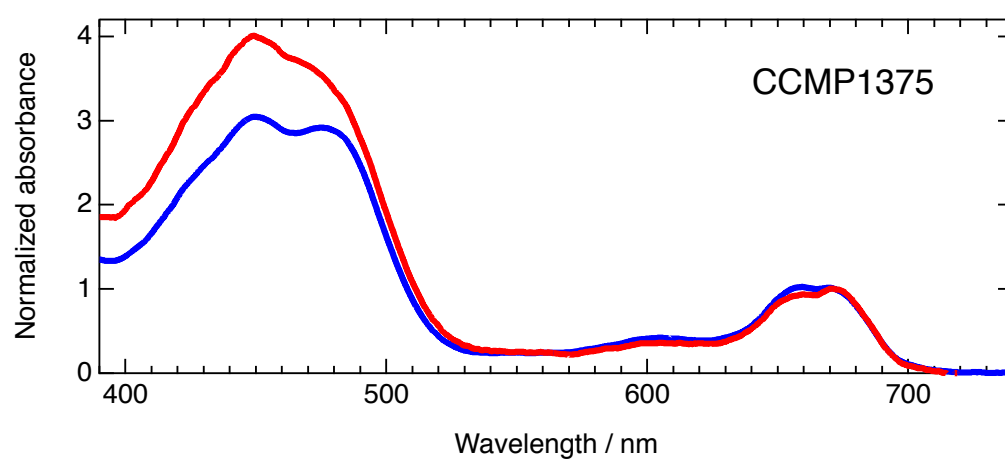


Figure 4-3. Steady-state absorption spectra of *Prochlorococcus* CCMP1375 grown under a blue LED (blue line) or red LED (red line) after growth under a red LED.

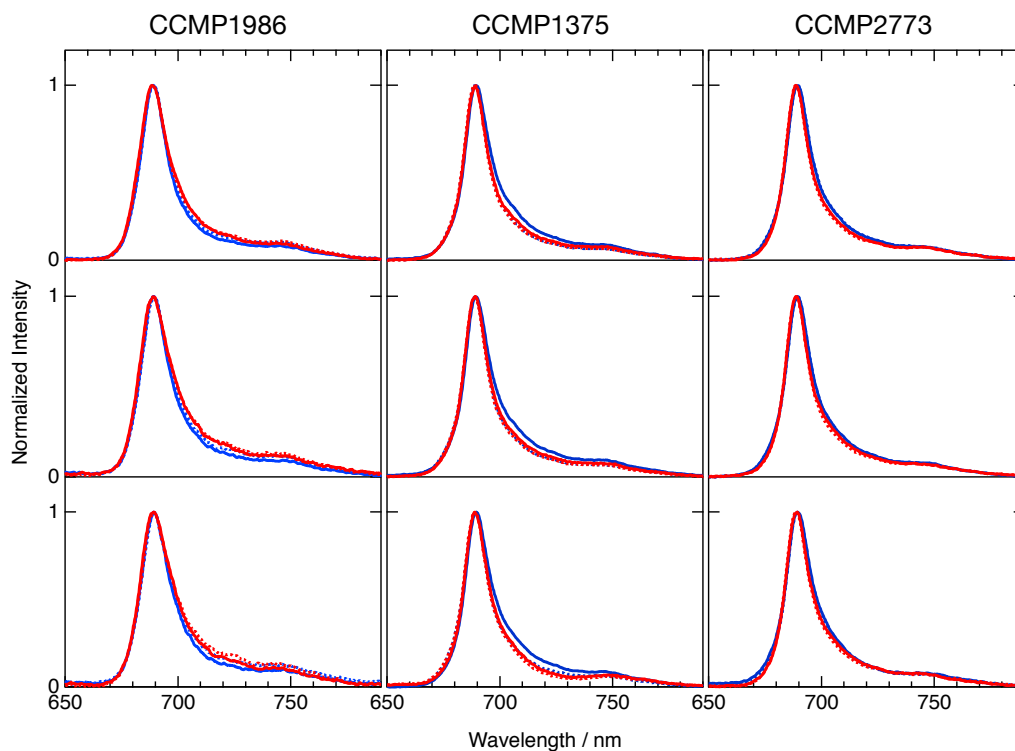


Figure 4-4. Steady-state fluorescence spectra of the three strains of *Prochlorococcus*, CCMP1986 (left), CCMP1375 (middle), and CCMP2773 (right), grown under various light conditions. The blue and red lines represent cells grown under blue and red LEDs, respectively. The solid and dotted lines represent cells grown under LEDs with higher and lower intensities, respectively. The excitation wavelength is 445 nm (top), 475 nm (middle), and 500 nm (bottom).

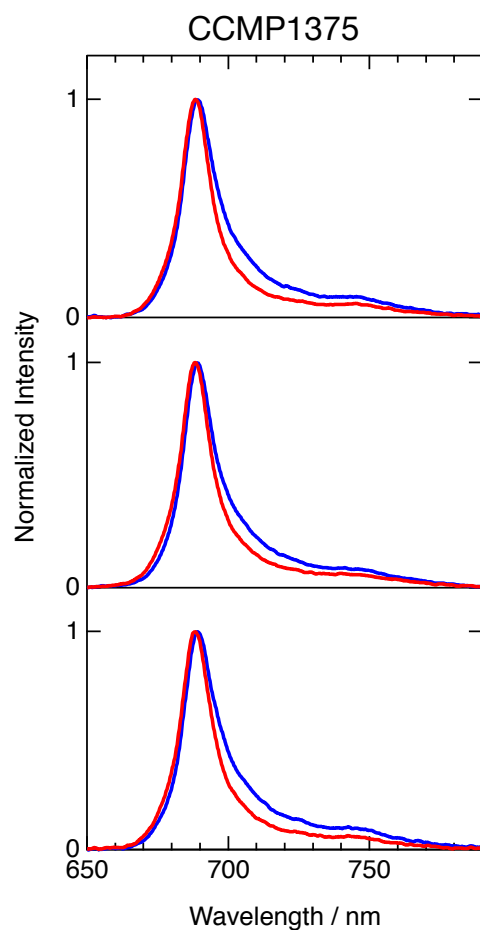


Figure 4-5. Steady-state fluorescence spectra of *Prochlorococcus* CCMP1375 grown under a blue LED (blue line) or red LED (red line) after growth under a red LED. The excitation wavelength is 445 nm (top), 475 nm (middle), and 500 nm (bottom).

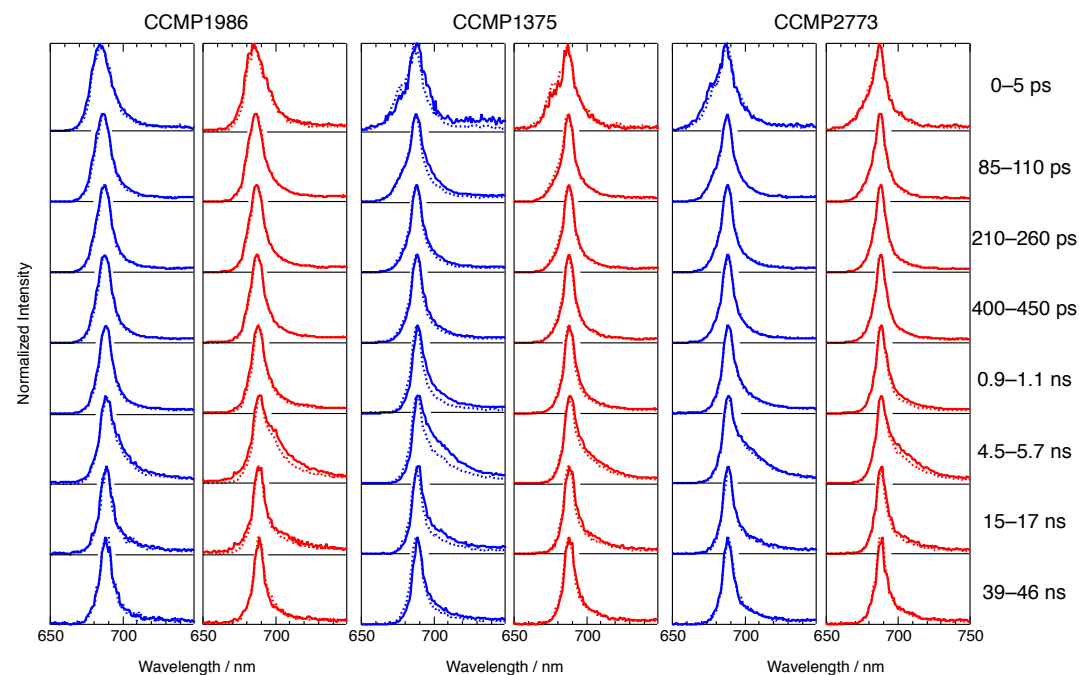


Figure 4-6. Time-resolved fluorescence (TRF) spectra of the three strains of *Prochlorococcus*, CCMP1986 (left), CCMP1375 (center), and CCMP2773 (right), grown under various light conditions. The blue and red lines represent cells grown under blue and red LEDs, respectively. The solid and dotted lines represent cells grown under LEDs with higher and lower intensities, respectively.

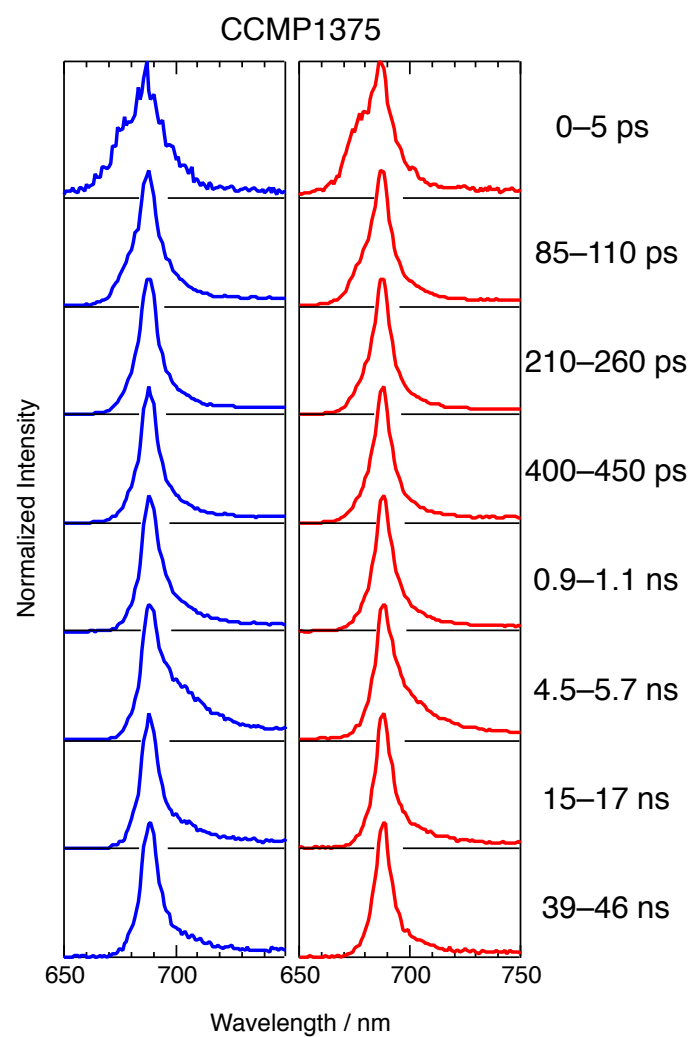


Figure 4-7. Time-resolved fluorescence (TRF) spectra of *Prochlorococcus* CCMP1375

grown under a blue LED (blue line) or red LED (red line) after growth under a red LED.

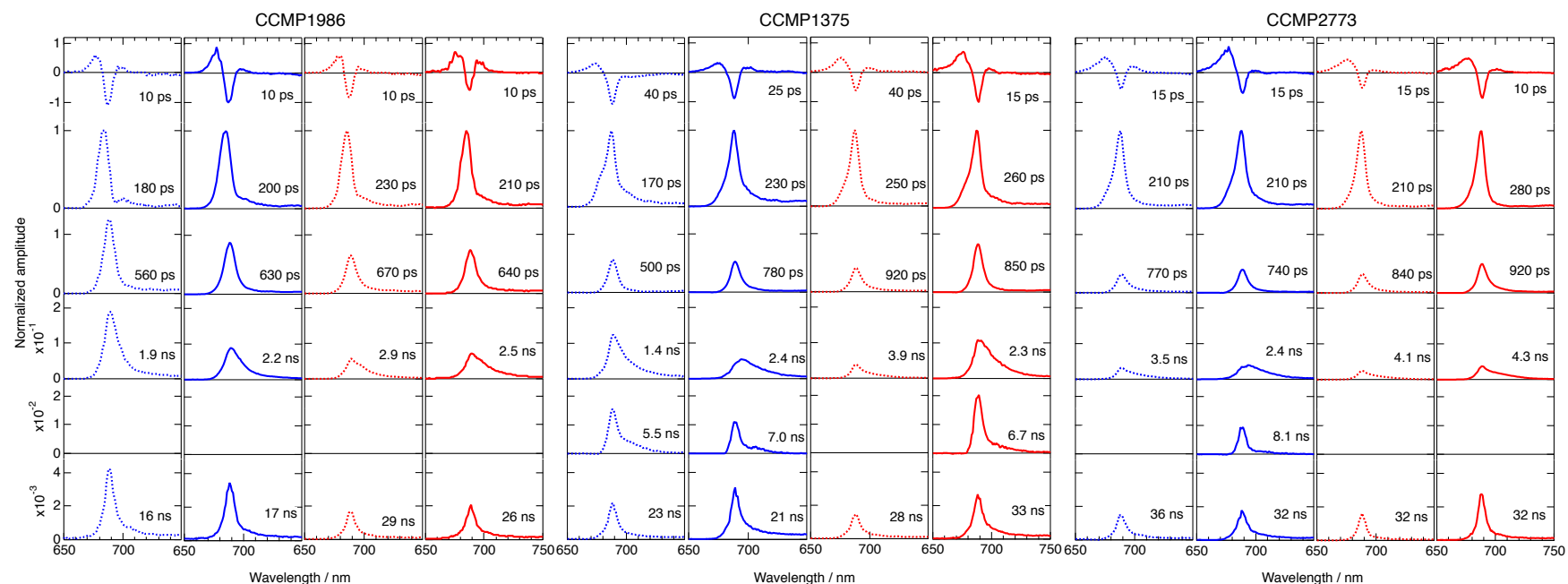


Figure 4-8. Fluorescence decay-associated (FDA) spectra of the three strains of *Prochlorococcus*, CCMP1986 (left), CCMP1375 (center), and CCMP2773 (right), obtained by global analysis. The blue and red lines indicate cells grown under blue and red LEDs, respectively. The solid and dotted lines represent cells grown under LEDs with higher and lower intensities, respectively.

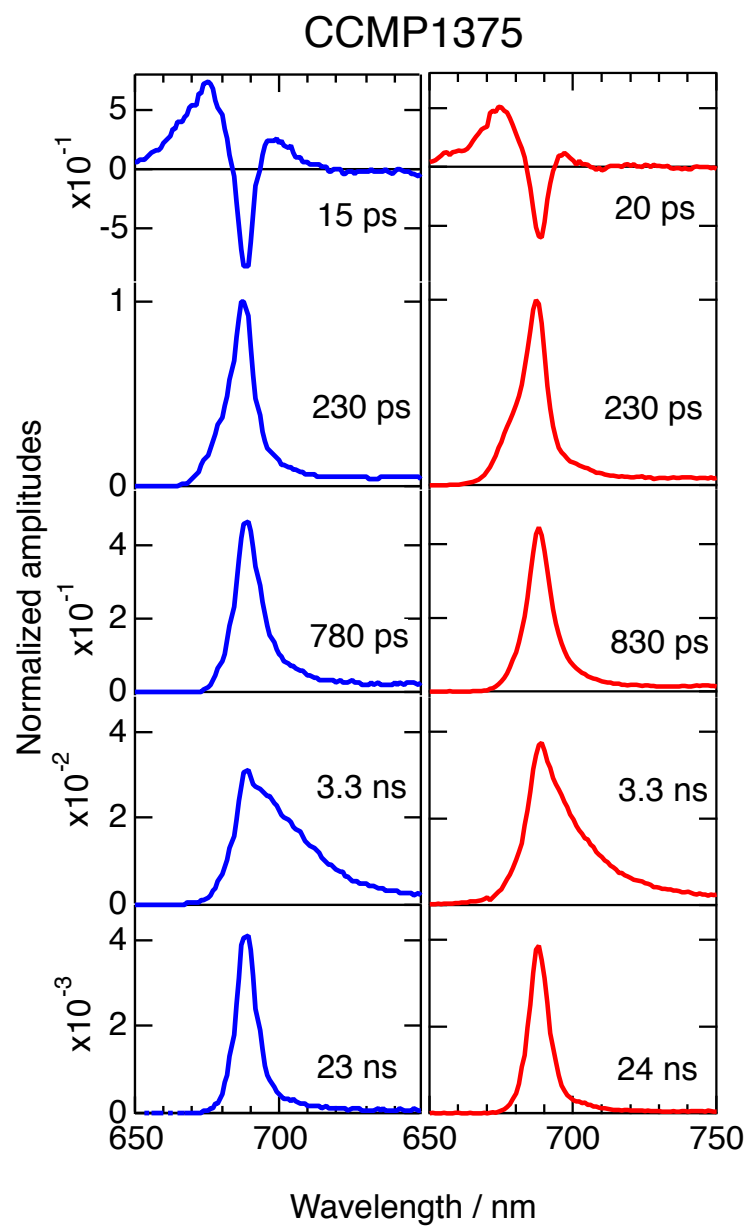


Figure 4-9. Fluorescence decay-associated (FDA) spectra of *Prochlorococcus* CCMP1375 grown under a blue LED (blue line) or red LED (red line) after growth under a red LED.

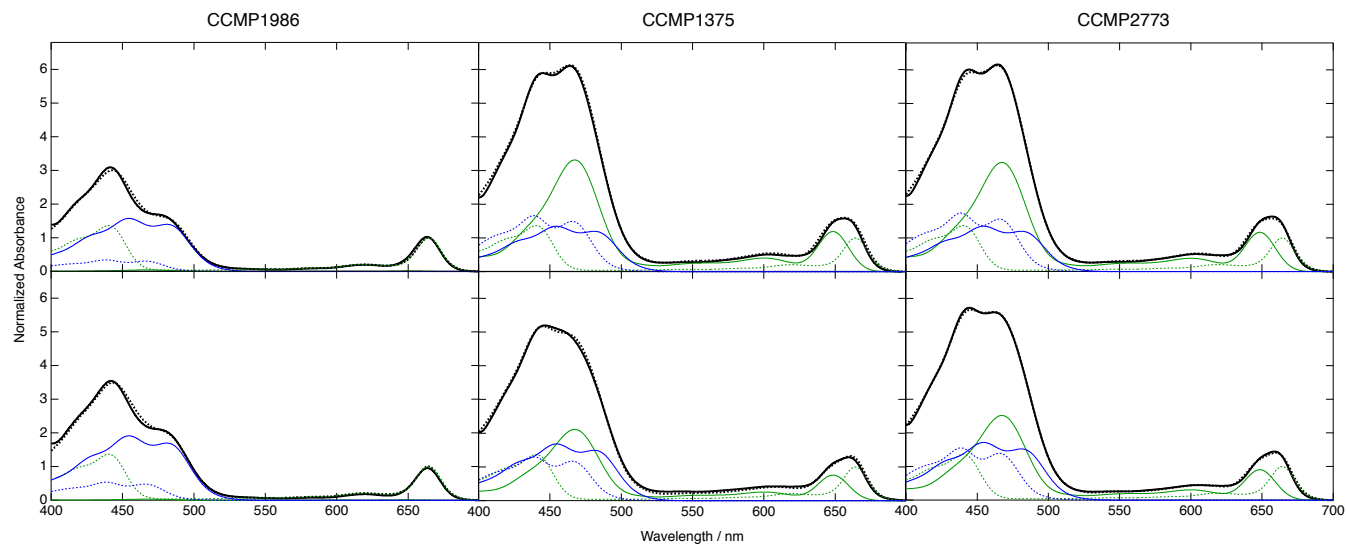


Figure 4-10.

Absorption spectra of the pigment extraction by acetone from the higher-light-grown cells of the three strains of *Prochlorococcus* at 293 K. The top windows are for the blue-LED-grown cells, while the bottom windows are for the red-LED-grown cells. The spectra (black solid lines) were analyzed with the components corresponding to the absorption spectra of DV-Chl *a* (green dotted line), DV-Chl *b* (green solid line), α -carotene (blue dotted line), and zeaxanthin (blue solid line). The black dotted lines are the resulting curve of the analysis. The absorbances were normalized at the Qy peak of DV-Chl *a* components.

Table 4-1. Relative pigment amount ratio of the extraction from the higher red light (R40 or R4)-grown cells to the higher blue light (B40 or B4)-grown cells of the three strains of *Prochlorococcus* at 293 K. The numbers on the table are the amount ratio of each pigment to DV-Chl *a*.

	DV-Chl <i>a</i>	DV-Chl <i>b</i>	α -Carotene	Zeaxanthin
CCMP1986	1.0	ca. 7	1.6	1.2
CCMP1375	1.0	0.64	0.78	1.3
CCMP2773	1.0	0.78	0.89	1.3

5. General Discussion

5.1 Comparison between Chl *b*-containing cyanobacteria and the other Chl *b*-containing organisms

When the light-harvesting functions of the (DV-) Chl *b*-containing cyanobacteria are compared to the other Chl *b*-containing photosynthetic organisms, they have common characteristics that (DV-) Chl *b* transfers the excitation energy to (DV-) Chl *a* with high efficiency observed as the time constants of sub-ps. *Prochlorococcus* showed the fast excitation energy transfer from DV-Chl *b* to DV-Chl *a* with the time constants of 0.68–1.0 ps, while the LHC in green algae and land plants showed the excitation energy transfer from Chl *b* to Chl *a* with time constants of 750–800 fs (Chloroplast from *Codium fragile*) or 535–750 fs (LHCII from *Arabidopsis thaliana*).^{5,6} For *Prochloron* and *Prochlorothrix*, the ultrafast excitation energy transfer process was not observed, because the time resolution of the measurement system was not enough to capture the process, and because the relative amount of Chl *b* in the organisms are much smaller. However, no clear fluorescence signal from Chl *b* was

observed in the ps-ns measurement, which indicates the ultrafast excitation energy transfer in less than a few ps may occur from Chl *b* to Chl *a*.

A difference between the Chl *b*-containing cyanobacteria and the other Chl *b*-containing photosynthetic organisms is the types and relative amounts of Car they have. *Prochloron* accumulates β -carotene and zeaxanthin. *Prochlorothrix* accumulates β -carotene, zeaxanthin, and trace amount of α -carotene.^{52,71} *Prochlorococcus* accumulates α -carotene and zeaxanthin. For the excitation energy transfer process, the characteristic process from Car S_1 to DV-Chl *a* Q_y was observed in *Prochlorococcus*, by the fs TRF measurement. Green algae and land plants has the higher Chl/Car amount ratio than *Prochlorococcus*, therefore it is difficult to observe the excitation energy transfer process from Car S_1 (especially of the main Car lutein) to Chl Q_y in LHCII. However, it is probable that this energy transfer occurs because the length of the conjugated double bond of lutein is the same to that of α -carotene.^{16,100}

In the point of energetically interaction between two photosystems, *Prochloron* showed direct excitation energy transfer from PSII to PSI, while *Prochlorothrix* and *Prochlorococcus* did not show the phenomenon. Chl *b*-containing

higher plant *Arabidopsis thaliana* is reported to have PSI-PSII complex and PSII directly transfer the excitation energy to PSI.⁹⁸ The difference may be because of the structure of the supercomplexes. Green algae and land plants have the LHC-PS supercomplex, and based on the structure reported, the LHC antenna proteins does not surround the PSI and PSII complex completely.⁹⁹ On the other hand, Pcb antenna in Chl *b*-containing cyanobacteria surrounds PSI trimer. *Prochlorothrix*, *Prochlorococcus* CCMP1375 and *Prochlorococcus* CCMP2773 are reported to have Pcb-PSI supercomplex with 18 Pcb protein which completely surrounds the PSI trimer.^{54,95,102} In that case, the direct excitation energy transfer from PSII to PSI seems difficult. On the other hand, *Prochloron* is reported to have not-complete Pcb-PSI supercomplex which lacks some of the Pcb, or is has bare PSI trimer.⁵⁵ Between the PSII and PSI without the complete antenna ring, the excitation energy transfer may occur.

5.2 The characteristics of the Chl *b*-containing cyanobacteria under the different light conditions

The excitation energy transfer processes in cyanobacteria *Prochloron*, *Prochlorothrix*, and *Prochlorococcus* under different light conditions are investigated in this work. As observed in the TRF and FDA spectra, when these three organisms are higher-light-adapted (not-shaded habitat for *Prochloron* in hospite; 30 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for *Prochlorothrix*; 40 or 20 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for *Prochlorococcus*), the interaction between Pcb and photosystems are weaker, compared to the lower-light-adapted cells (shaded habitat for *Prochloron* in hospite; 4 or 2 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for *Prochlorococcus*). The phenomenon behind the difference in the interaction between Pcb and photosystems are not clear here, but there are some possible explanations. In the case of IsiA antenna ring around PSI, the size of the supercomplex can be modified. IsiA can make single or double ring complex around PSI.¹⁰³ Though the double ring is not reported for Pcb, if the size of the antenna ring changes, the time constants for the excitation energy transfer from Pcb to photosystems can change. Another possibility is the effect of the quenching by Cars in Pcb. As mentioned in Chap.

3, the S_1 energy level of α -carotene and zeaxanthin is close to that of Chl Qy. Especially, the energy level of zeaxanthin S_1 should be close to that of Chl *a* Qy in Pcb, and zeaxanthin is possessed by *Prochloron*, *Prochlorothrix*, and *Prochlorococcus*. For the FDA spectra of *Prochloron* and *Prochlorothrix* (Fig. 2-4), the first time constants are dependent on the cells, and classified as the faster ones (11 or 18 ps) and the slower ones (50 or 51 ps). It seems that the signals from several donors and acceptors are mixed in the FDA spectra, but the amplitudes of the decay and rise regions are comparable to each other. This indicate that the size of the antenna ring can be the main reason for the difference. On the other hand, for *Prochlorococcus* (especially CCMP1375), time constants and the relative amplitudes of decay and rise signals are different between the samples. The PSI signal from *Prochlorococcus* cells are weak as a whole, therefore the quenching by zeaxanthin might be affecting the difference in the time constants and spectral shapes.

Furthermore, for *Prochloron*, the interaction between the two photosystems judged from the delayed fluorescence was stronger for the shaded cells. That can be for sharing the energy between the photosystems under the limited light condition.

Synechocystis sp. PCC6803 adapted in short time to PSI-selective red light weakened the interaction between the photosystems, because of the direct excitation of PSI.³⁸ *A. platensis* grown under different light conditions, FL-light and several colors of LEDs, showed the difference in the interaction between the photosystems.³⁵ The cells grown by the light which Chl *a* does not absorb, especially yellow light, indicated the more efficient energy transfer between the two photosystems. *C. merolae* grown under the different color LED also showed the similar behavior that the relative intensity of PSI delayed fluorescence increased under yellow light and decreased under red light.⁴¹ The modification of the interaction between the two photosystems in *Prochloron* may be close to that in the other organisms in that it occurs under limitation of PSI excitation.

Not only the excitation energy transfer process, but the pigment composition in *Prochlorococcus* was modified under the different light conditions. When compared between the blue and red LED-grown cells of the DV-Chl *b*-rich strains (CCMP2773 and especially CCMP1375), the amount of DV-Chl *b* and α -carotene was smaller under the red LED. On the other hand, for CCMP1986, the amount of α -carotene was larger under the red LED. For all the strains, the amount of zeaxanthin was larger under the

red LED. *F. diplosiphon* is known to change the composition of phycobiliprotein under the different light color, accumulating the antenna protein which can absorb the culture light.^{33,34} This behavior seems to close to that of *Prochlorococcus*, especially DV-Chl *b*-rich CCMP1375 and CCMP2773. These two strains may be possible to modify the types of Pcb antenna depending on the light conditions, as mentioned in Chapter 4. This is contrary to the cases of some other organisms. *C. reinhardtii* modify the relative amount of the two photosystems under the light conditions of PSII- or PSI-selective, in order to compensate the uneven excitation of the two photosystems.³⁹ Under the PSII-selective light, relative amount of PSI was larger. *A. platensis* grown under a fluorescent lamp and LEDs of different colors, which resulted in modification of the ratios of the pigment content.³⁵ Under the light that PBS does not absorb (blue and far-red lights), the relative amount of PBS was larger and the excitation energy transfer in PBS was indicated to be faster. Furthermore, when *A. platensis* was grown under blue light, quenching was observed in the PSI. In the case of *Prochlorococcus*, the amount of zeaxanthin, which may work for quenching,^{96,97} was increased under red

light. That can be because the blue light is the main component of the natural habitat of *Prochlorococcus*.

When comparing between the Chl *b*-containing cyanobacteria under similar light conditions, there are some common and different points. As discussed in Chapter 2, *Prochlorothrix* showed comparable time constants and spectral shapes to *Prochloron* under higher light conditions in FDA spectra (Fig. 2-4). The light intensity for the growth of *Prochlorothrix* was 30 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$, which is close to the light intensity for the growth of *Prochlorococcus* CCMP1986 (20 or 40 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$). However, the spectral shapes and time constants of FDA spectra of *Prochlorothrix* and *Prochlorococcus* CCMP1986 are different in some points. Time constants in the fastest FDA spectra are significantly different, which means the interaction between Pcb and photosystems are different, and PSI signal was marginal in *Prochlorococcus*. In the previous works, *Prochlorothrix* under the light intensity around 30 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ is reported to accumulate only PcbC, while *Prochlorococcus* CCMP1986 accumulates only PcbA.^{54,71,91} Furthermore, the phylogenetic studies showed that *Prochlorothrix*, *Prochlorococcus*, and *Prochloron* are not especially close to each other among the other

cyanobacteria or green algae.⁹²⁻⁹⁴ This indicate that these three organisms have different strategies for adapting themselves to the light conditions of their habitat, though they all have Pcb antenna and (DV-) Chl *b*.

Conclusion

In this doctoral dissertation, the light-harvesting functions in the Chl *b*-containing cyanobacteria, *Prochloron*, *Prochlorothrix*, and *Prochlorococcus*, inhabiting or grown under the different light conditions are investigated. *Prochloron*, which cannot live by itself and are harboring to different types of ascidians, are under the different light conditions by the effects of the light conditions at the habitat and the body of the host ascidians. The *Prochloron* cells adjusting the energetically binding between PSII and PSI depending the light conditions. On the other hand, the free-living *Prochlorothrix* cells grown without shade effect exhibited weak energetically bonding between the photosystems, and the characteristics are close to one of the *Prochloron* in hospite. DV-Chl-containing *Prochlorococcus*, grown under white fluorescent lamp, showed efficient excitation energy transfer between DV-Chl *b* and DV-Chl *a*, independent of the strains. Furthermore, especially in the lower light-adapted strains, Cars also function as the significant antenna pigment that transfers the excitation energy to DV-Chl *a*. When the *Prochlorococcus* cells were grown under the blue LED, which corresponds to the light conditions of DV-Chl *b*-rich strains, pigment composition

seemed not changed significantly compared to the cells grown under white fluorescent lamp. On the other hand, when the cells are grown under the red LED, the amount of DV-Chl *b* was reduced in the DV-Chl *b*-rich strains, and that of zeaxanthin increased in the specific strain CCMP1375. Under both color of LEDs, the time constants of excitation energy transfer between DV-Chl *a* did not change significantly.

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References

- (1) Blankenship, R.E.: Photosynthetic Pigments: Structure and Spectroscopy. In *Molecular Mechanisms of Photosynthesis, 2nd Edition*; Blankenship, R.E.; Wiley-Blackwell, Oxford, **2014**.
- (2) Rüdiger, W.; Schoch, S.: Chlorophylls. In *Plant Pigments*; Goodwin, T.W., Ed.; Academic Press Ltd., London, **1988**; pp 1–59.
- (3) Kleima, F.J.; Gradinaru, C.C.; Calkoen, F.; van Stokkum, I.H.M.; van Grondelle, R.; van Amerongen, H. Energy Transfer in LHCII Monomers at 77K Studied by Sub-Picosecond Transient Absorption Spectroscopy, *Biochemistry* **1997**, 36, 15262–15268.
- (4) Agarwal, R.; Krueger, B.P.; Scholes, G.D.; Yang, M.; Yom, J.; Mets, L.; Fleming, G.R. Ultrafast Energy Transfer in LHC-II Revealed by Three-Pulse Photon Echo Peak Shift Measurements, *J. Phys. Chem. B* **2000**, 104, 2908–2918.
- (5) Akimoto, S.; Yamazaki, I.; Murakami, A.; Takaichi, S.; Mimuro, M. Ultrafast Excitation Relaxation Dynamics and Energy Transfer in the Siphonaxanthin-Containing Green Alga *Codium fragile*, *Chem. Phys. Lett.* **2004**, 390, 45–49.

- (6) Akimoto, S.; Yokono, M.; Ohmae, M.; Yamazaki, I.; Tanaka, A.; Higuchi, M.; Tsuchiya, T.; Miyashita, H.; Mimuro, M. Ultrafast Excitation Relaxation Dynamics of Lutein in Solution and in the Light-Harvesting Complexes II Isolated from *Arabidopsis thaliana*, *J. Phys. Chem. B* **2005**, 109, 12612–12619.
- (7) Papagiannakis, E.; van Stokkum, I.H.M.; Fey, H.; Büchel, C.; van Grondelle, R. Spectroscopic Characterization of the Excitation Energy Transfer in the Fucoxanthin-Chlorophyll Protein of Diatoms, *Photosyn. Res.* **2005**, 86, 241–250.
- (8) Akimoto, S.; Tomo, T.; Naitoh, Y.; Otomo, A.; Murakami, M.; Mimuro, M. Identification of a New Excited State Responsible for the in vivo Unique Absorption Band of Siphonaxanthin in the Green Alga *Codium fragile*, *J. Phys. Chem. B* **2007**, 111, 9179–9181.
- (9) Gildenhoff, N.; Herz, J.; Gundermann, K.; Büchel, C.; Wachtveitl, J. The Excitation Energy Transfer in the Trimeric Fucoxanthin-Chlorophyll Protein from *Cyclotella meneghiniana* Analyzed by Polarized Transient Absorption Spectroscopy, *Chem. Phys.* **2010**, 373, 104–109.
- (10) Akimoto, S.; Teshigahara, A.; Yokono, M.; Mimuro, M.; Nagao, R.; Tomo, T.

Excitation Relaxation Dynamics and Energy Transfer in Fucoxanthin-Chlorophyll *a/c* Protein Complexes, Probed by Time-Resolved Fluorescence, *Biochim. Biophys. Acta* **2014**, 1837, 1514–1521.

(11) Govindjee; Satoh, K.: Fluorescence Properties of Chlorophyll *b*- and Chlorophyll *c*-Containing Algae. In *Light Emission by Plants and Bacteria*; Govindjee; Ames, J.; Fork, D.C., Eds.; Academic Press, Massachusetts, **1986**; pp 497–537.

(12) Mimuro, M.; Hirayama, K.; Uezono, K.; Miyashita, H.; Miyachi, S. Uphill Energy Transfer in a Chlorophyll *d*-Dominating Oxygenic Photosynthetic Prokaryote, *Acaryochloris marina*, *Biochim. Biophys. Acta* **2000**, 1456, 27–34.

(13) Schenderlein, M.; Çetin, M.; Barber, J.; Telfer, A.; Schlodder, E. Spectroscopic Studies of the Chlorophyll *d* Containing Photosystem I from the Cyanobacterium, *Acaryochloris marina*, *Biochim. Biophys. Acta* **2008**, 1837, 1400–1408.

(14) Tomo, T.; Shinoda, T.; Chen, M.; Allakhverdiev, S.I.; Akimoto, S. Energy Transfer Processes in Chlorophyll *f*-Containing Cyanobacteria Using Time-Resolved Fluorescence Spectroscopy on Intact Cells, *Biochim. Biophys. Acta* **2014**, 1837, 1484–1489.

- (15) Akimoto, S.; Shinoda, T.; Chen, M.; Allakhverdiev, S.I.; Tomo, T. Energy Transfer in the Chlorophyll *f*-Containing Cyanobacterium, *Halomicronema hongdechloris*, Analyzed by Time-Resolved Fluorescence Spectroscopies, *Photosynth. Res.* **2015**, 125, 115–122.
- (16) Takaichi, S. Carotenoids in Algae: Distributions, Biosyntheses and Functions, *Mar. Drugs* **2011**, 9, 1101–1118.
- (17) Kana, T.M.; Glibert, P.M.; Goericke, R.; Welschmeyer, N.A. Zeaxanthin and β -Carotene in *Synechococcus* WH7803 Respond Differently to Irradiance. *Limnol. Oceanogr.* **1998**, 33, 1623–1627.
- (18) Masamoto, K.; Zsiros, O.; Gombos, Z. Accumulation of Zeaxanthin in Cytoplasmic Membranes of the Cyanobacterium *Synechococcus* sp. Strain PCC 7942 Grown Under High Light Condition. *J. Plant Physiol.* **1999**, 155, 136–138.
- (19) Wilson, A.; Ajlani, G.; Verbavatz, J.-M.; Vass, I.; Kerfeld, C.A.; Kirilovsky, D. A soluble carotenoid protein involved in phycobilisome-related Energy Dissipation in cyanobacteria. *Plant Cell* **2006**, 18, 992–1007.

- (20) McColl, R. Cyanobacterial Phycobilisomes, *J. Struct. Biol.* **1998**, 124, 311–334.
- (21) Grossman, A.R.; Schaefer, M.R.; Chiang, G.G.; Collier, J.L. The Phycobilisome, a Light-Harvesting Complex Responsive to Environmental Conditions, *Microbiol. Rev.* **1993**, 57, 725–749.
- (22) Andrizhiyevskaya, E.G.; Schwabe, T.M.E.; Germano, M.; D’Haene, S.; Kruip, J.; van Grodelle, R.; Dekker, J.P. Spectroscopic Properties of PSI-IsiA Supercomplexes from the Cyanobacterium *Synechococcus* PCC 7942, *Biochim. Biophys. Acta* **2002**, 265–272.
- (23) Burnap, R.L.; Troyan, T.; Sherman, L.A. The Highly Abundant Chlorophyll-Protein Complex of Iron-Deficient *Synechococcus* sp. PCC7942 (CP43’) is Encoded by the *isiA* Gene, *Plant Physiol.* **1993**, 103, 893–902.
- (24) Bibby, T.S.; Nield, J.; Barber, J. Iron Deficiency Induces the Formation of an Antenna Ring Around Trimeric Photosystem I in Cyanobacteria, *Nature* **2001**, 412, 743–745.
- (25) Boekema, E.J.; Hifney, A.; Yakushevskaya, A.E.; Pitrowski, M.; Keegstra, W.; Berry,

S.; Michel, K.P.; Pistorius, E.K.; Krup, J. A Giant Chlorophyll-Protein Complex Induced by Iron Deficiency in Cyanobacteria, *Nature* **2001**, 412, 745–748.

(26) Brakemann, T.; Schlörmann, W.; Marquardt, J.; Nolte, M.; Rhiel, E. Association of Fucoxanthin Chlorophyll *a/c*-Binding Polypeptides with Photosystems and Phosphorylation in the Centric Diatom *Cyclotella cryptica*, *Protist* **2006**, 157, 463–475.

(27) Caffari, S.; Kouřil, R.; Kreiche, S.; Boekema, E.J.; Croce, R. Functional Architecture of Higher Plant Photosystem II Supercomplex, *EMBO J.* **2009**, 28, 3052–3063.

(28) Kargul, J.; Nield, J.; Barber, J. Three-Dimensional Reconstruction of a Light-Harvesting Complex I-Photosystem I (LHCI-PSI) Supercomplex from the Green Alga *Chlamydomonas reinhardtii*. Insights into light harvesting for PSI, *J. Biol. Chem.* **2003**, 278, 16135–16141.

(29) Bumba, L.; Prášil, O.; Vácha, F. Antenna Ring around Trimeric Photosystem I in Chlorophyll *b* Containing Cyanobacterium *Prochlorothrix hollandica*, *Biochim. Biophys. Acta* **2005**, 1708, 1–5.

- (30) Lepetit, B.; Volke, D.; Szabó, M.; Hoffmann, R.; Garab, G.; Willhelm, C.; Goss, R. Spectroscopic and Molecular Characterization of the Oligomeric Antenna of the Diatom *Phaeodactylum tricornutum*, *Biochemistry* **2007**, 46, 9813–9822.
- (31) Nagao, R.; Yokono, M.; Akimoto, S.; Tomo, T. High Excitation Energy Quenching in Fucoxanthin Chlorophyll *a/c*-Binding Protein Complexes from the Diatom *Chaetoceros gracilis*, *J. Phys. Chem. B* **2013**, 117, 6888–6895.
- (32) Tanaka, K.; Iida, S.; Takaichi, S.; Mimuro, M.; Murakami, A.; Akimoto, S. Excitation Relaxation Dynamics and Energy Transfer in Pigment-Protein Complexes of Dinoflagellate, Revealed by Ultrafast Fluorescence Spectroscopy, *Photosyn. Res.* **2016**, 130, 183–191.
- (33) Yokono, M.; Akimoto, S.; Koyama, K.; Tsuchiya, T.; Mikuro, M. Energy Transfer Processes in *Gloeobacter violaceus* PCC 7421 that Possesses Phycobilisomes with a Unique Morphology, *Biochim. Biophys. Acta* **2008**, 1777, 55–65.
- (34) Stowe-Evans, E.L.; Kehoe, D.M.; Signal Transduction during Light-Quality Acclimation in Cyanobacteria: a Model System for Understanding

Phytochrome-Response Pathways in Prokaryotes, *Photochem. Photobiol. Sci.* **2004**, 3, 495–502.

(35) Akimoto, S.; Yokono, M.; Hamada, F.; Teshigahara, A.; Aikawa, A.; Kondo, A, Adaptaion of Light-Harvesting Systems of *Arthrospira platensis* to Light Conditions, Probed by Time-Resolved Fluorescence Spectroscopy, *Biochim. Biophys. Acta* **2012**, 1817, 1483–1489.

(36) Wang, C.Y.; Fu, C.C.; Liu, Y.C. Effects of Using Light-Emitting Diodes on the Cultivation of *Spirulina platensis*, *Biochem. Eng. J.* **2007**, 37, 21–25.

(37) Chen, H.B.; Wu, J.Y.; Wang, C.F.; Fu, C.C.; Shieh, C.J.; Chen, C.I.; Wang, C.Y. Liu, Y.C. Modeling on Chlorophyll a and Phycocyanin Production by *Spirulina platensis* Under Various Light-Emitting Diodes, *Biochem. Eng. J.* **2010**, 53, 52–56.

(38) Akimoto, S.; Yokono, M.; Yokono, E.; Aikawa, S.; Kondo, A. Short-Term Light Adaptation of a Cyanobacterium, *Synechocystis* sp. PCC6803, Probed by Time-Resolved Fluorescence Spectroscopy, *Plant Physiol. Biochem.* **2014**, 81, 149–154.

(39) Melis, A.; Murakami, A.; Nemson, J.A.; Aizawa, K.; Ohki, K.; Fujita, Y.

Chromatic Regulation in *Chlamydomonas reinhardtii* Alters Photosystem

Stoichiometry and Improves the Quantum Efficiency of Photosynthesis, *Photosyn. Res.*

1996, 47, 253–265.

(40) Fu, W.; Guðmundsson, Ó, Pagilia, G.; Herjólfsson, G.; Andrésson, Ó.S.; Pálsson,

B.Ø.; Brynjólfsson, S. Enhancement of Carotenoid Biosynthesis in the Green Microalga

Dunaliella salina with Light-Emitting Diodes and Adaptive Laboratory Evolution, *Appl.*

Microbiol. Biotechnol. **2013**, 97, 2395–2403.

(41) Ueno, Y.; Aikawa, S.; Kondo, A.; Akimoto, S. Light Adaptation of the Unicellular

Red Alga, *Cyanidinoschyzon merolae*, probed by time-resolved fluorescence

spectroscopy, *Photosynth. Res.* **2015**, 125, 211–218.

(42) Schulze, P.S.C.; Barreira, L.A.; Pereira, H.G.C.; Perales, J.A.; Varela, J.C.S. Light

Emitting Diodes (LEDs) Applied to Microalgal Production, *Trends in Biotech.* **2014**, 32,

422–430.

(43) Kühl, M.; Larkum, A.W.D.; The Microenvironment and Photosynthetic

Performance of *Prochloron* sp. in Symbiosis with Didemnid Ascidians, In *Cellular*

origin and life in extreme habitats; J. Seckbach; Kluwer Academic Publishers, Dordrecht, **2002**, Vol. 3, 273–290.

(44) Hirose, E.; Neilan, B.A.; Schmidt, E.W.; Murakami, A. Enigmatic life and evolution of *Prochloron* and related cyanobacteria inhabiting colonial ascidians, In *Handbook on Cyanobacteria*; Gault, P.M.; Marler, H.J.; Nova Science Publishers, New York, **2009**, 161–189.

(45) Hamada, F.; Yokono, M.; Hirose, E.; Murakami, A.; Akimoto, S. Excitation Energy Relaxation in a Symbiotic Cyanobacterium, *Prochloron didemni*, Occurring in Coral-Reef Ascidians, and in a Free-Living Cyanobacterium, *Prochlorothrix hollandica*, *Biochim. Biophys. Acta* **2012**, 1817, 1992–1997.

(46) Hess, W.R.; Partensky, F.; van der Staay, G.W.M.; Garcia-Fernandez, J.M.; Borner, T.; Vault, D. Coexistence of Phycoerythrin and a Chlorophyll *a/b* Antenna in a Marine Prokaryote, *Proc. Natl. Acad. Sci. USA* **1996**, 93, 11126–11130.

(47) Seewaldt, E.; Stackbrandt, E. Partial Sequence of 16S Ribosomal RNA and the Phylogeny of *Prochloron*, *Nature* **1982**, 295, 618–620.

- (48) Palenik, B.; Haselkorn, R. Multiple Evolutionary Origins of Prochlorophytes, the Chlorophyll *b*-Containing Prokaryotes, *Nature* **1992**, 355, 265–266.
- (49) Urbach, E.; Robertson, E.L.; Chisholm, S.W. Multiple Evolutionary Origins of Prochlorophytes within the Cyanobacterial Radiation, *Nature* **1992**, 355, 267–276.
- (50) Palenik, B.; Swift, H. Cyanobacterial Evolution and Prochlorophyte Diversity as Seen in DNA-Dependent RNA Polymerase Gene Sequences, *J. Phycol.* **1996**, 32, 638–646.
- (51) La Roche, J.; van der Staay, G.W.M.; Partensky, F.; Ducret, A.; Aebershold, R.; Li, R.; Golden, S.S.; Hiller, R.G.; Wrench, P.M.; Larkum, A.W.D. et al. Independent Evolution of the Prochlorophyte and Green Plant Chlorophyll *a/b* Light Harvesting Proteins, *Proc. Natl. Acad. Sci. U. S. A.* **1996**, 93, 15244–15248.
- (52) Bibby, T.S.; Nield, J.; Chen, M.; Larkum, A.W.D.; Barber, J. Structure of a Photosystem II Supercomplex Isolated from *Prochloron didemni* Retaining its Chlorophyll *a/b* Light-Harvesting System, *Proc. Natl. Acad. Sci. U. S. A.* **2003**, 100, 9050–9054.

- (53) van der Staay, G.W.M.; Yurkova, N.; Green, B.R. The 38 kDa Chlorophyll *a/b* Protein of the Prokaryote *Prochlorothrix hollandica* is Encoded by a Divergent *pcb* gene, *Plant Mol. Biol.* **1998**, 36, 709–716.
- (54) Bibby, T.S.; Mary, I.; Nield, J.; Partensky, F.; Barber, J. Low-Light-Adapted *Prochlorococcus* Species Possess Specific Antennae for each Photosystem, *Nature* **2003**, 424, 1051–1054.
- (55) Münchhoff, J.; Hirose, E.; Maruyama, T.; Sunairi, M.; Burns, B.P.; Neilan, B.A. Host Specificity and Phylogeography of the Prochlorophyte *Prochloron* sp., an Obligate Symbiont in Didemnid Ascidians, *Environ. Microbiol.* **2007**, 9, 890–899.
- (56) Cox, G.C. Comparison of *Prochloron* from different hosts. I. Structural and Ultrastructural Characteristics, *New Phytologist* **1986**, 104, 429–445.
- (57) Mimuro, M.; Murakami, A.; Tomo, T.; Tsuchiya, T.; Watanabe, K.; Yokono, M.; Akimoto, S. Molecular Environments of Divinyl Chlorophylls in *Prochlorococcus* and *Synechocystis*: Differences in Fluorescence Properties with Chlorophyll Replacement, *Biochim. Biophys. Acta* **2011**, 1807, 471–481.
- (58) Takaichi, S.; Mochimaru, M.; Uchida, H.; Murakami, A.; Hirose, E.; Maoka, T.;

Tsuchiya, T.; Mimuro, M. Opposite Chilarity of α -Carotene in Unusual Cyanobacteria with Unique Chlorophylls, *Acaryochloris* and *Prochlorococcus*, *Plant Cell Physiol.* **2012**, 53, 1881–1888.

(59) Hamada, F.; Murakami, A.; Akimoto, S.; Comparative Analysis of Ultrafast Excitation Energy-Transfer Pathways in Three Strains of Divinyl Chlorophyll *a/b*-Containing Cyanobacterium, *Prochlorococcus marinus*, *J. Phys. Chem. B* **2015**, 119, 15593–15600.

(60) Croce, R.; ven Amerongen, H. Natural Strategies for Photosynthetic Light Harvesting, *Nat. Chem. Bil.* **2014**, 10, 492–501.

(61) Partensky, F.; Hoepffner, N.; Li, W.K.W.; Ulloa, O.; Vaultot, D. Photoacclimation of *Prochlorococcus* sp. (Prochlorophyta) Strains Isolated from the Forth Atlantic and the Mediterranean Sea, *Plant Physiol.* **1993**, 101, 285–296.

(62) Boichemko, V. A.; Pinevich, A. A.; Stadnichuk, I. N. Association of Chlorophyll *a/b*-Binding Pcb Proteins with Photosystems I and II in *Prochlorothrix hollandica*, *Biochim. Biophys. Acta* **2007**, 1767, 801–806.

- (63) Moore, L.R.; Goericke, R.; Chisholm, S.W. Comparative Physiology of *Synechococcus* and *Prochlorococcus*; Influence of Light and Temperature on Growth, Pigments, Fluorescence and Absorptive Properties, *Mar. Ecol. Prog. Ser.* **1995**, 116, 259–275.
- (64) Yokono, M. Murakami, A.; Akimoto, S. Excitation Energy Transfer between Photosystem II and Photosystem I in Red Algae: Larger Amounts of Phycobilisome Enhance Spillover, *Biochim. Biophys. Acta* **2011**, 1807, 847–853.
- (65) Mimuro, M.; Yokono, M.; Akimoto, S. Variations on Photosystem I Properties in the Primordial Cyanobacterium *Gloeobacter violaceus* PCC 7421, *Photochem. Photobiol.* **2010**, 86, 62–69.
- (66) Arzhantsev, S.; Maroncelli, M. Design and Characterization of a Femtosecond Fluorescence Spectrometer Based on Optical Kerr Gating, *Appl. Spectrosc.* **2005**, 59, 206–220.

- (67) Hirose, E.; Hirose, M.; Neilan, B.A. Localization of Symbiotic Cyanobacteria in the Colonial Ascidian *Trididemnum miniatum* (Didemnidae, Ascidiacea), *Zool. Sci.* **2006**, 23, 435–442.
- (68) Hirose, E.; Hikabayashi, S.; Hori, K.; Kasai, F.; Watanabe, M.M. UV Protection in the Photosymbiotic Ascidian *Didemnum molle* Inhabiting Different Depth, *Zool. Sci.* **2006**, 23, 57–63.
- (69) Hirose, E.; Maruyama, T. What are the benefits in the ascidian-*Prochloron* symbiosis ?, In *Endosymbiosis and Eukaryotic Organelles. Proceedings of the 8th International Congress on Endocytobiology and Symbiosis (Endocytobiology VIII)*; Sugiura, M.; Oelmueller, R.; Obokata, J.; **2004**, 15, 51–62.
- (70) Hirose, E.; Ohtsuka, K.; Ishikura, M.; Maruyama, T. Ultraviolet Absorption in Ascidian Tunic and Ascidian-*Prochloron* Symbiosis, *J. Mar. Biol. Ass. U. K.* **2004**, 84, 789–794.
- (71) Herbstová, M.; Litvín, R.; Gardian, Z.; Komenda, J.; Vácha, F. Localization of Pcb Antenna Complexes in the Photosynthetic Prokaryote *Prochlorothrix hollandica*, *Biochim. Biophys. Acta* **2010**, 1797, 89–97.

- (72) Bricaud, A.; Allali, K.; Morel, A.; Marie, D.; Veldhuis, M.J.W.; Partensky, F.; Vault, D. Divinyl Chlorophyll *a*-Specific Absorption Coefficients and Absorption Efficiency Factors for *Prochlorococcus marinus*: Kinetics of Photoacclimation, *Mar. Ecol. Prog. Ser.* **1999**, 188, 21–32.
- (73) Dufresne, A.; Salanoubat, M.; Partensky, F.; Artiguenave, F.; Axmann, I.M.; Barbe, V.; Duprat, S.; Galperin, M.Y.; Koonin, E.V.; Gall, F.L. et al. Genome Sequence of the Cyanobacterium *Prochlorococcus marinus* SS120, a Nearly Minimal Oxyphototrophic Genome, *Proc. Natl. Acad. Sci. U. S. A.* **2003**, 100, 10020–10025.
- (74) Akimoto, S.; Mimuro, M. Application of Time-Resolved Polarization Fluorescence Spectroscopy in the Femtosecond Range to Photosynthetic Systems, *Photochem. Photobiol.* **2007**, 83, 163–170.
- (75) Shedbalkar, V.P.; Rebeiz, C.A. Chloroplast Biogenesis: Determination of the Molar Extinction Coefficients of Divinyl Chlorophyll *a* and *b* and Their Pheophytins, *Anal. Biochem.* **1992**, 207, 261–266.
- (76) Nakayama, K.; Mimuro, M.; Nishimura, Y.; Yamazaki, I.; Okada, M. Kinetic Analysis of Energy Transfer Processes in LHC II Isolated from the Siphonous Green

Alga, *Bryopsis maxima* with Use of Picosecond Fluorescence Spectroscopy, *Biochim.*

Biophys. Acta **1994**, 1188, 117–124.

(77) Nakayama, K.; Mimuro, M. Chlorophyll Forms and Excitation Energy Transfer

Pathways in Light-harvesting Chlorophyll *a/b*-protein Complexes Isolated from the

Siphonous Green Alga, *Bryopsis maxima*, *Biochim. Biophys. Acta* **1994**, 1184, 103–110.

(78) Kandori, H.; Sasabe, H.; Mimuro, M. Direct Determination of a Lifetime of the S₂

State of β -Carotene by Femtosecond Time-Resolved Fluorescence Spectroscopy, *J. Am.*

Chem. Soc. **1994**, 116, 2671–2672.

(79) Itoh, T. Lowest Singlet Excited State and Spectroscopy of α -Carotene, *Chem. Phys.*

Lett. **2011**, 505, 96–99.

(80) Frank, H.A.; Bautista, J.A.; Josue, J.S.; Young, A.J. Mechanism of

Nonphotochemical Quenching in Green Plants: Energies of the Lowest Excited Singlet

States of Violaxanthin and Zeaxanthin, *Biochemistry* **2000**, 39, 2831–2837.

(81) Nagae, H.; Kakitani, T.; Katoh, T.; Mimuro, M. Calculation of the Excitation

Transfer Matrix Elements Between the S₂ or S₁ State of Carotenoid and the S₂ or S₁

State of Bacteriochlorophyll, *J. Chem. Phys.* **1993**, 98, 8012–8023.

- (82) Jahns, P.; Holtzwarth, A.R. The Role of Xanthophyll Cycle and of Lutein in Photoprotection of Photosystem II, *Biochim. Biophys. Acta* **2012**, 1817, 182–193.
- (83) Zuo, P.; Li, B.X.; Zhao, X.H.; Wu, Y.S.; Ai, X.C.; Zhang, J.P.; Li, L.B.; Kuang, T.Y. Ultrafast Carotenoid-to-Chlorophyll Singlet Energy Transfer in the Cytochrome *b₆f* Complex from *Bryopsis corticalans*, *Biophys. J.* **2006**, 90, 4145–4154.
- (84) Polívka, T.; van Stokkum, I.H.M.; Zigmantas, D.; van Grondelle, R.; Sundström, V.; Hiller, R.G. Energy Transfer in the Major Intrinsic Light-Harvesting Complex from *Amphidinium carterae*, *Biochemistry* **2006**, 45, 8516–8526.
- (85) Koyama, Y.; Kuki, M.; Andersson, P.O.; Gillbro, T. Singlet Excited States and the Light-Harvesting Function of Carotenoids in Bacterial Photosynthesis, *Photochem. Photobiol.* **1996**, 63, 243–256.
- (86) Frank, H.A.; Cogdell, R.J. Carotenoids in Photosynthesis, *Photochem. Photobiol.* **1996**, 63, 257–264.
- (87) Hiller, R.G.: Carotenoids as Components of Light-Harvesting Proteins of Eukaryotic Algae. In *The Photochemistry of Carotenoids*; Frank. H.A.; Young, A.J.; Britton, G.; Cogdell, R.J., Eds.; Kluwer Academic Publishers, Dordrecht, Netherland,

1999; pp 81–98.

(88) Akimoto, S; Yokono, M.; Yokono, E.; Aikawa, S.; Kondo, A.; Short-Term Light Adaptation of a Cyanobacterium, *Synechosystis* sp. PCC 6803, Probed by Time-Resolved Fluorescence Spectroscopy, *Plant. Physiol. Biochem.* **2014**, 81, 149–154.

(89) Mimuro, M.; Akimoto, S.; Yamazaki, I.; Miyashita, H.; Miyachi, S. Fluorescence Properties of Chlorophyll *d*-Dominating Prokaryotic Alga, *Acaryochloris marina*: Studies Using Time-Resolved Fluorescence Spectroscopy on Intact Cells, *Biochim. Biophys. Acta* **1999**, 1412, 37–46.

(90) Bullerjahn, G.S.; Matthijs, H.C.P.; Mur, L.R.; Sherman, D.M. Chlorophyll-Protein Composition of the Thylakoid Membrane from *Prochlorothrix hollandica*, a Prokaryote Containing Chlorophyll *b*, *Eur. J. Biochem.* **1987**, 168, 295–300.

(91) Chen, M.; Hiller, R.G.; Howe, C.J.; Larkum, A.W.D. Unique Origin and Lateral Transfer of Prokaryotic Chlorophyll-*b* and Chlorophyll-*d* Light-Harvesting Systems, *Mol. Biol. Evol.* **2005**, 22, 21–28.

- (92) Chen, M.; Zhang, Y.; Blankenship, R.E. Nomenclature for Membrane-Bound Light-Harvesting Complexes of Cyanobacteria, *Photosyn. Res.* **2008**, 95, 147–154.
- (93) Moore, L.R.; Rocap, G.; Chisholm, S.W. Physiology and Molecular Phylogeny of Coexisting *Prochlorococcus* Ecotypes, *Nature* **1998**, 393, 464–467.
- (94) Partensky, F.; Hess, W.R.; Vaulot, D. *Prochlorococcus*, A Marine Photosynthetic Prokaryote of Global Significance, *Microbiol. Mol. Biol. Rev.* **1999**, 63, 106–127.
- (95) Bibby, T.S.; Nield, J.; Partensky, F.; Barber, J. Antenna Ring Around Photosystem I, *Nature* **2001**, 413, 590.
- (96) Ballittari, M.; Dall'Osto, L.; Morosinotto, T.; Bassi, R. Contrasting Behavior of Higher Plant Photosystems I and II Antenna Systems during Acclimation, *J. Biol. Chem.* **2007**, 282, 8947–8958.
- (97) Demmig-Adams, B. Survey of Thermal Energy Dissipation and Pigment Composition in Sun and Shade Leaves, *Plant Cell Physiol.* **1998**, 39, 474–482.

- (98) Yokono, M.; Takabayashi, A.; Akimoto, S.; Tanaka, A. A Megacomplex Composed of Both Photosystem Reaction Centres in Higher Plants, *Nat. Commun.* **2015**, 6, 6675.
- (99) Wobbe, L.; Bassi, R.; Kruse, O. Multi-Level Light Capture Control in Plants and Green Algae, *Trends Plant Sci.* **2016**, 21, 55–68.
- (100) Walla, P.J.; Linden, P.A.; Ohta, K.; Fleming, G.R. Excited-State Kinetics of the Carotenoid S₁ State in LHCII and Two-Photon Excitation Spectra of Lutein and β -Carotene in Solution: Efficient Car S₁→Chl Electronic Energy Transfer via Hot S₁ States?, *J. Phys. Chem. A* **2002**, 106, 1909–1916.
- (101) Bumba, L.; Prasil, O.; Vacha, F. Antenna Ring Around Trimeric Photosystem I in Chlorophyll *b* Containing Cyanobacterium *Prochlorothrix hlandica*, *Biochim. Biophys. Acta* **2005**, 1708, 1–5.
- (102) Kouřil, R.; Arteni, A.A.; Lax, J.; Yermenko, N.; D’Haene, S.; Ronger, M.; Matthijs, H.C.P.; Dekker, J.P.; Boekema, E.J. Structure and Functional Role of

Supercomplexes of IsiA and Photosystem I in Cyanobacterial Photosynthesis, *FEBS*

Lett. **2005**, 579, 3253–3257.