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Fluorescence properties of the chlorophyll *d*-dominated cyanobacterium *Acaryochloris* sp. strain Awaji

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Abstract

Fluorescence properties of the newly discovered chlorophyll (Chl) *d*-dominated cyanobacterium, *Acaryochloris* sp. strain Awaji were examined by a combination of steady state and the time-resolved fluorescence measurements. *Acaryochloris* sp. strain Awaji, in contrast to *Acaryochloris marina* MBIC11017, exhibited photosystem (PS) II fluorescence at 745 nm at room temperature and at 750 nm at −196 °C with a few Chl *d* components in the shorter wavelength region of the maxima. PS I fluorescence was not detected by steady state measurements but was by time-resolved spectroscopy as a transient component. The results clearly indicated diversity in the antenna systems in the genus *Acaryochloris* that contains Chl *d* as major pigments. Delayed fluorescence was observed only in the Chl *a* fluorescence region, consistent with *A. marina*. This strongly suggested that the primary electron donor for the PS II reaction center should be Chl *a*, which is identical to all other oxygenic photosynthetic organisms.

Keywords: Chlorophyll *d*; Energy transfer; Photosynthesis; Time-resolved fluorescence; *Acaryochloris*

1. Introduction

Chlorophylls play important roles, such as light harvesting in antenna systems and electron transfer in reaction centers in photosynthetic organisms [1]. Most oxygenic photosynthetic organisms contain chlorophyll (Chl) *a* as predominant pigments, and Chl *b*, Chl *c* and Chl *d* are also present in addition to Chl *a* in oxygenic photosynthetic organisms (Scheme 1) [2]. It is known that Chl *b* molecules work as antenna pigment in green algae and higher plants, and Chl *c*, in brown algae, diatom and dinoflagellates [2]. Since Q_y bands of Chl *b* and Chl *c* locate at shorter wavelengths than that of Chl *a*, Chl *b* and Chl *c* transfer excitation energy to Chl *a* with high efficiencies [3–7]. Although the diversity of antenna systems containing Chl *b* and Chl *c* molecules is well studied [8,9], little is known in respect to antenna systems having Chl *d* molecules because only one genus is recognized as a Chl *d*-dominated organism. *Acaryochloris marina* is a cyanobacterium that contains Chl *d* as a major Chl pigment, along with a small amount of Chl *a* [10]. In *A. marina*, Chl *d* molecules work as antenna pigments in photosystem (PS) I and in PS II [11] and as the primary electron donor of PS I [12], whereas Chl *a* molecules are the primary electron donor of PS II [13,14]. Under a high light condition, additional Chl *a* molecules are accumulated in cells; therefore, it has been suggested that the additional Chl *a* molecules function as a quencher of Chl *d* [14].

In 2004, Murakami et al. discovered a Chl *d*-dominated cyanobacterium, *Acaryochloris* sp. strain Awaji (hereafter referred as *Acaryochloris* Awaji) on the surface of a macrophytic red alga *Ahnfeltiopsis flabelliformis*, and concluded that the real producer of 'Chl *d* in red algae' was the cyanobacterium that attached to the surface of red algae [15]. *Acaryochloris* Awaji was classified to the same clade as that of *A. marina* MBIC11017 (hereafter referred as *A. marina*) based on the 16S

Abbreviations: APC, allophycocyanin; Chl, chlorophyll; DF, delayed fluorescence; FR, far-red; PC, phycocyanin; PS, photosystem; TRFS, time-resolved fluorescence spectra

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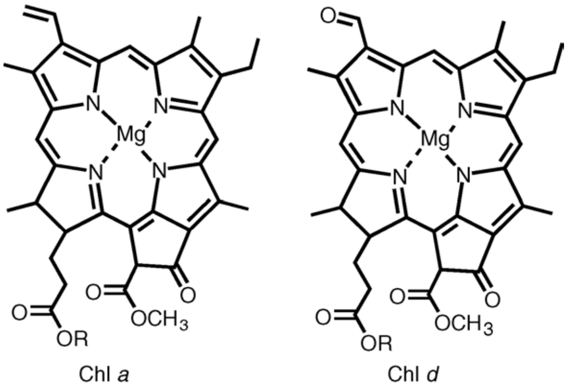

Scheme 1. Molecular structures of Chl *a* and Chl *d*. R stands for phytol chain.

rRNA sequence [15]; the difference between them was only 12 out of 1258 bases in the conserved region of 16S rRNA. Compared with *A. marina*, *Acaryochloris* Awaji showed a red-shifted fluorescence peak by spectroscopy under a microscope [15]: 715 nm for *A. marina* and 729 nm for *Acaryochloris* Awaji. This suggests a variation in the fluorescence properties of the two strains of the genus *Acaryochloris*. Therefore in the present study, we examined the fluorescence properties of *Acaryochloris* Awaji by means of steady state and picosecond time-resolved fluorescence spectroscopy. We discussed energy transfer and charge recombination (electron transfer) processes in photosystems having Chl *d* molecules as a major pigment.

2. Materials and methods

2.1. Algal culture

Acaryochloris sp. strain Awaji was isolated from a red alga *A. flabelliformis* [15] and grown in IMK-medium (Nihon Pharmaceutical Co., Japan) [11,13] based on natural seawater under 12-h light:12-h dark condition. Culture bottles were settled on the shelf in an incubator and illuminated from the top. Air was not supplied. Cells were grown under two light conditions; one for growth under far-red light (hereafter referred as FR-light) to excite predominantly PS I, and the other for growth under light longer than the yellow region (hereafter referred as W-light) to excite both PS I and PS II. For the former, a glass plate filter (R-70, Toshiba, Japan) was used in combination with a far-red enriched fluorescent lamp (FL20S FR-74, Toshiba Electronics, Japan). For the latter, a yellow acryl filter (Sumipex #200, Sumitomo Chemical, Japan) was used in combination with a normal type fluorescent lamp (FL40SW, NEC, Japan). Temperature for growth was 22 °C. The growth rate of *Acaryochloris* Awaji was very low; doubling time was longer than 5 days. Therefore, an intermediate(s) for pigment biosynthesis might accumulate, as is reported for other cyanobacteria.

A. marina MBIC11017 was used as a reference. Cells were grown in IMK-medium based on artificial seawater under incandescent light. Air was continuously supplied through a filter (Millex, Millipore, USA). Growth temperature was 22 °C.

Cells were harvested from culture bottles and used without any treatment.

2.2. Steady state and time-resolved spectroscopy

Steady state absorption and fluorescence spectra were recorded under a microscope (Olympus BX50) using a light-guided multi-channel photodiode array detector (PMA-11, Hamamatsu Photonics, Japan). Excitation wavelength for fluorescence spectra was 435 nm (bright line of mercury lamp). Steady state fluorescence spectrum at liquid nitrogen temperature (−196 °C) was measured with a fluorescence spectrometer (Hitachi 850, Japan), in combination with a custom-made Dewar bottle [13]. Time-resolved fluorescence spectra (TRFS) and fluorescence decay curves were measured with a picosecond time-correlated single-photon counting system [13,16]. The light source was a Ti:Sapphire laser (Spectra-Physics Tsunami, USA), and the second harmonic of the Ti:Sapphire laser generated by a BBO crystal (425 nm) was used for excitation pulses. We employed a microchannel plate photomultiplier (Hamamatsu R3809, Japan) as a detector, combined with a monochromator (Nikon P-250, Japan). Measurements of TRFS at −196 °C were carried out with a custom-made Dewar system [13]. For low temperature fluorescence spectroscopy, polyethylene glycol (average molecular weight, 3350, Sigma) was added to obtain homogeneous ice (final concentration, 15%). Fluorescence lifetimes were estimated by an iterative convolution calculation, and the time resolution was 3 ps.

3. Results

3.1. Steady state absorption and fluorescence spectra

Fig. 1 shows absorption spectra of *Acaryochloris* Awaji at room temperature measured under a microscope. Cells grown under W-light showed the absorption maximum at approximately 706 nm with several additional components in both wavelength regions of the maximum, i.e. 695, 714 and 738 nm. In the Soret region, the maximum was located at 460 nm, which came from Chl *d*. Absorption of carotenoids was clearly observed at approximately 500 nm. These features were retained in cells grown under FR-light; an additional feature was a small bump at 650 nm that may be ascribed to phycobiliproteins. A long tail in the Q_y region of Chl *d* was common to both types of cells that indicate multiple Chl *d* bands. This was striking difference from that of *A. marina* together with a blue-shifted maximum.

Fig. 2 shows fluorescence spectra of *Acaryochloris* Awaji at room temperature measured under a microscope. When grown under FR-light, the fluorescence peak was detected at 745 nm with a shoulder at approximately 728 nm. The third peak at 710 nm was cryptic but most probably present. Contrarily, when grown under W-light, at least two peaks were detected at approximately 710 and 744 nm; the 730 nm band was not clearly resolved but its presence was likely. To this extent, three fluorescence bands were common to both types of cells. A fluorescence band at approximately 675 nm was small but clear in the W-light grown cells; this was a clear difference between the two types of cells.

It is known that in cyanobacteria, a fluorescence peak at room temperature comes from Chl *a* and is detected at approximately

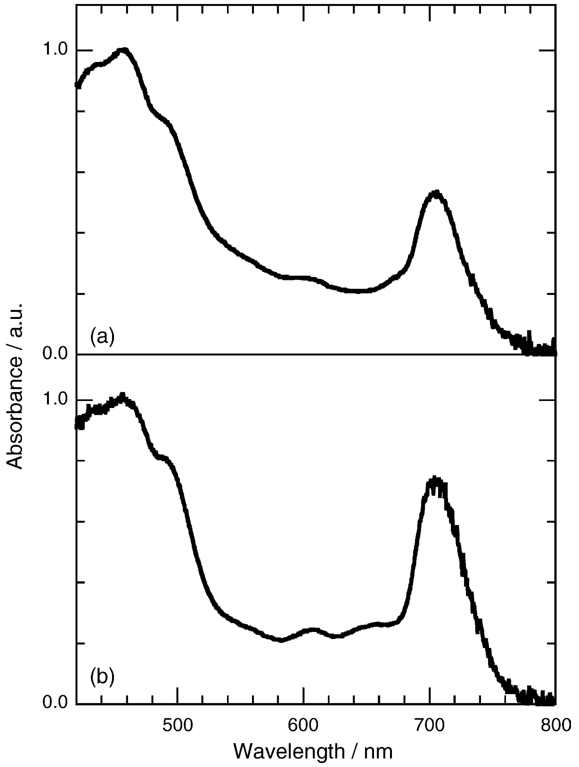

Fig. 1. Absorption spectra of *Acaryochloris* Awaji measured by microspectrophotometry: (a) cells grown under W-light and (b) those under FR-light. Cells are sticky toward each other; therefore, the absorption spectrum was measured only under a microscope with use of a light guide and a detector (Hamamatsu Photonics, PMA-11, Japan).

685 nm. The magnitude of variation is small, usually within a few nanometers. This shows a conserved molecular architecture of the PS II in cyanobacteria, and this is also applicable to Chl *a*/*b* organisms (green algae and higher plants). In the case of *Acaryochloris* Awaji, a large red-shift of the fluorescence maximum was observed from 685 nm in other cyanobacteria to 745 nm, and was assigned to replacement of Chl *a* with Chl *d*, which was not surprising. However, the presence of plural PS II fluorescence components was unique.

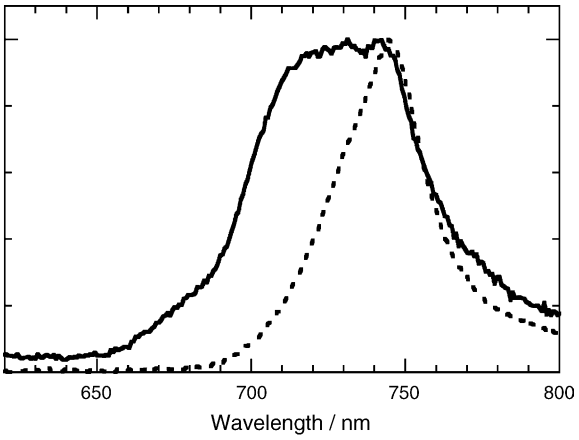

Fig. 2. Steady state fluorescence spectra of *Acaryochloris* Awaji at room temperature measured by microspectrophotometry: cells grown under W-light (solid line) and those under FR-light (broken line). Excitation wavelength was 435 nm.

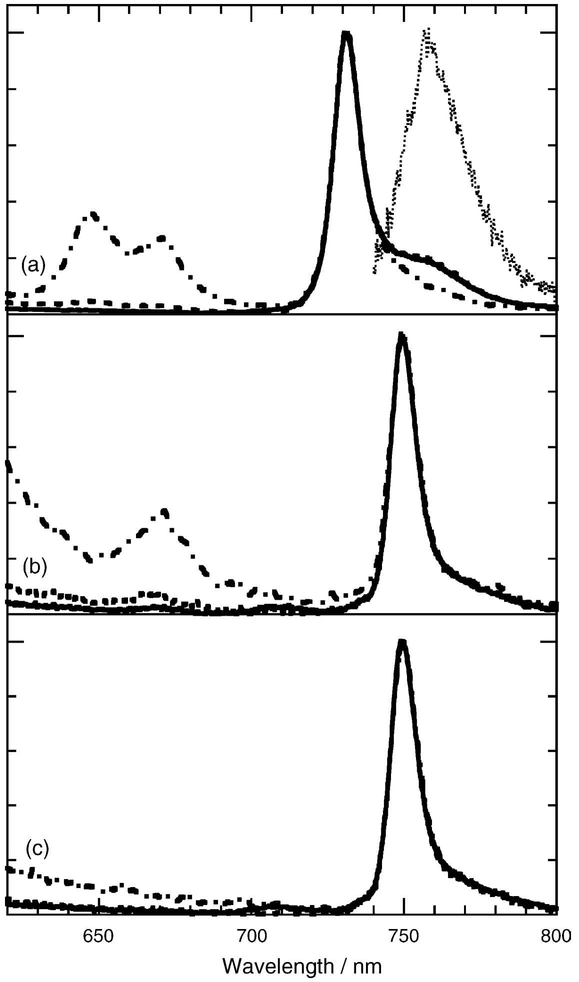

Fig. 3. Steady state fluorescence spectra at −196 °C: (a) *A. marina*, (b) *Acaryochloris* Awaji grown under W-light and (c) those grown under FR-light. Excitation wavelength was 470 nm (solid line), 495 nm (broken line) and 550 nm (dashed dotted line). A difference spectrum is shown as a dotted line in (a).

Fig. 3 shows fluorescence spectra of *A. marina* and *Acaryochloris* Awaji cells at −196 °C. Three peaks were located at 647, 670 and 731 nm for *A. marina* cells, and two peaks at 670 and 750 nm for *Acaryochloris* Awaji cells. Besides these peaks, weak bands were observed around 710 and 760 nm for the former, and around 647, 710 and 770 nm for the latter. For *A. marina* cells, the 731 nm band was assigned to fluorescence from PS II, and the 760 nm band to PS I fluorescence [13]. This assignment was confirmed by excitation wavelength dependence of fluorescence intensities. The 760 nm band of *A. marina* cells decreased in its relative intensity to the 731 nm band upon excitation of phycocyanin (PC) at 550 nm, whereas it increased upon excitation of carotenoid (495 nm) or Chl *d* (470 nm). Therefore, it can be concluded that the 760 nm band of *A. marina* cells is due to fluorescence from Chl *d* molecules that do not accept excitation energy from phycobiliproteins, i.e. Chl *d* molecules in PS I. The PS I fluorescence band of *A. marina* cells, which was obtained as a difference spectrum between the spectra upon the phycobiliproteins and Chl *d* excitations, showed a broad band at 760 nm (Fig. 3a). In addition to a decrease in the fluorescence intensity of the 760 nm band, the 647 nm, and the 670 nm bands increased in their intensities, indicating that these bands are responsible for the fluorescence of PC or allophycocyanin (APC).

The most intense band was shifted to the red by 19 nm in the spectrum of the FR-light grown *Acaryochloris* Awaji cells (750 nm), compared with that of *A. marina* cells (731 nm), and the 750 nm maximum was close to the *A. marina* PS I fluorescence (760 nm) band rather than the *A. marina* PS II band. However, the 750 nm band was assigned to the PS II fluorescence because it exhibited strong fluorescence upon excitation by PC. The 750 nm band has a tail to the red side, and the relative intensity of the 750 nm band and this tail did not depend on the excitation wavelength. This is in striking contrast to the fluorescence behavior of *A. marina* cells. No fluorescence band that could be responsible for PS I fluorescence was observed by steady state excitation for *Acaryochloris* Awaji cells, however, its presence was suggested by TRFS (see Section 3.2).

At room temperature, fluorescence spectra of *Acaryochloris* Awaji varied according to the light condition for growth (Fig. 2). However, at −196 °C, there was a minor difference in spectra; the main fluorescence was observed at 750 nm for both types of cells, except for minor bands in the short-wavelength region of the maximum (Fig. 3).

3.2. *Time-resolved fluorescence spectra*

TRFS of *Acaryochloris* Awaji cells grown under W-light and FR-light conditions are depicted in Fig. 4a and b, respectively, after normalization to the maximum intensities in individual spectra. At the very beginning, both types of cells exhibited a peak at 745 nm with a broad band in the short-wavelength region; peaks were recognized at 718 and 734 nm. These three corresponded to the fluorescence bands detected at room temperature (Fig. 2) with a slight variation in wavelengths. When increasing the time delay, the blue side of the 745 nm band decreased in its intensity, resulting in a dynamic red-shift with a spectral narrowing. After approximately 400 ps, the maximum was located at 749 nm as observed in the steady state fluorescence spectra at −196 °C (Fig. 3), indicating that the Chl *d* molecules, which emit the 749 nm fluorescence, work as energy sinks in PS II of *Acaryochloris* Awaji. On the other hand, in the wavelength region longer than 750 nm, the relative intensities varied with time; a relative intensity at 778 nm decreased from 0.12 (92–120 ps) to 0.06 (3.8–4.3 ns) for the W-light grown *Acaryochloris* Awaji cells, and from 0.14 (92–120 ps) to 0.06 (3.8–4.3 ns) for the FR-light grown *Acaryochloris* Awaji cells. This behavior is consistent with TRFS of *A. marina* cells where the PS I band was recognized around 760 nm at 50 ps, and vanished at the later time stage [13]. Even though the PS I fluorescence of *Acaryochloris* Awaji cells was not detected in the steady state measurements (Fig. 3b), it was recognized in TRFS as a short-lived component with low intensity. In addition to these results, the spectra of the W-light grown *Acaryochloris* Awaji cells exhibited an additional peak at 670 nm. The relative intensity of the 670 nm band to that of the PS II band increased with time, suggesting that the 670 nm pigments do not contribute to energy transfer in PS II. After 15 ns, the FR-light grown *Acaryochloris* Awaji cells emitted fluorescence at approximately 630 and 670 nm; the latter corresponded to the component observed in the W-light grown cells, however, the origin of the former was not yet identified.

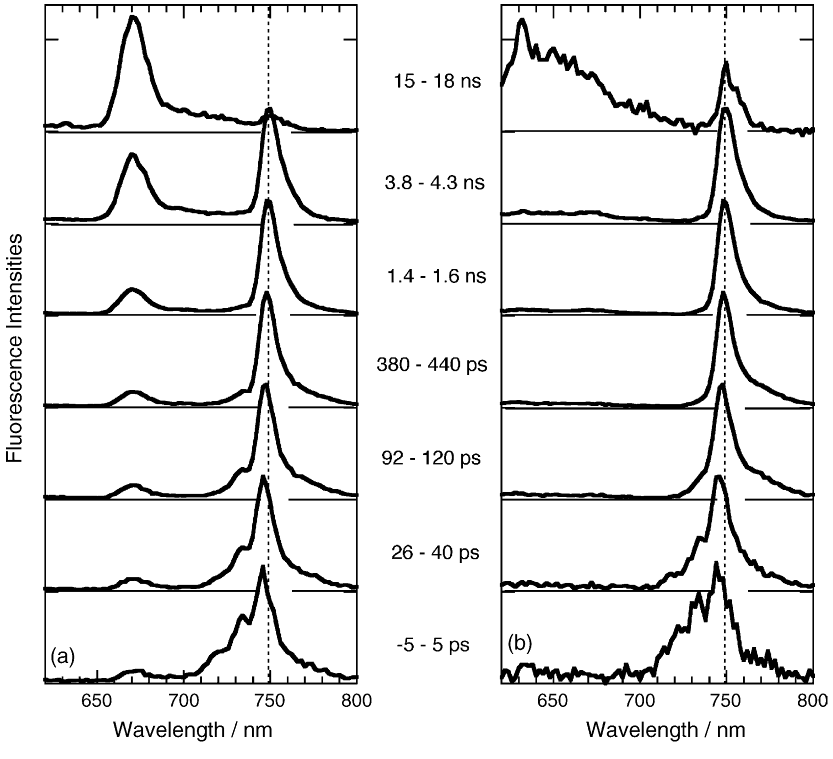

Fig. 4. Time-resolved fluorescence spectra of *Acaryochloris* Awaji at −196 °C: (a) cells grown under W-light conditions and (b) those grown under FR-light conditions. Excitation wavelength was 425 nm.

Table 1
Analyzed fluorescence lifetimes and their amplitudes for W-light and FR-light grown *Acaryochloris* Awaji cells at −196 °C

Sample	Wavelength (nm)	Lifetime (Amplitude)				
		(ps)	(ps)	(ns)	(ns)	(ns)
Acaryochloris Awaji W-light grown cells	670	–	300 (0.115)	4.33 (0.254)	7.80 (0.631)	–
	685	–	210 (0.200)	2.04 (0.133)	6.59 (0.619)	11.5 (0.048)
	720	35 (0.810)	290 (0.124)	2.27 (0.025)	7.03 (0.041)	–
	750	–	920 (0.402)	2.12 (0.564)	4.85 (0.034)	–
	778	30 (−0.557)	510 (0.683)	2.00 (0.298)	4.80 (0.019)	–
Acaryochloris Awaji FR-light grown cells	670	–	250 (0.287)	1.98 (0.288)	5.98 (0.425)	–
	685	–	400 (0.224)	1.76 (0.303)	5.22 (0.424)	12.8 (0.049)
	720	30 (0.917)	360 (0.046)	2.35 (0.022)	6.68 (0.015)	–
	750	30 (−0.801)	710 (0.251)	1.86 (0.656)	3.48 (0.093)	–
	778	40 (−0.956)	520 (0.715)	1.99 (0.252)	3.15 (0.033)	–

Excitation wavelength was 425 nm.

3.3. Fluorescence rise and decay kinetics

Fluorescence rise and decay curves of *Acaryochloris* Awaji cells were measured at several wavelengths typical to specific molecular species or spectral components of Chl *d* (Fig. 5). Lifetimes analyzed by convolution calculations are summarized in Table 1. A short time constant of 30–40 ps was resolved at longer than 720 nm, indicating a fast relaxation process among Chl *d* molecules. On the other hand, a long-lived component was resolved in fluorescence decay curves at 685 nm: 11.5 ns for the W-light grown cells and 12.8 ns for the FR-light grown cells. This was also the case in *A. marina* where a 15 ns component was resolved on the decay at 685 nm [13]. A clear difference in dynamics between *Acaryochloris* Awaji and *A. marina* cells was recognized in the wavelength region longer than the respective fluorescence maxima; the fluorescence kinetics of the W-light grown *Acaryochloris* Awaji cells at 778 nm and that of the FR-light grown *Acaryochloris* Awaji cells at 750 and 778 nm contained a rise component(s), whereas *A. marina* cells did not show any rise component longer than 3 ps in the wavelength regions examined [13]; 3 ps was a time resolution of our measuring system. The rise time of the 778 nm band for the W-light grown *Acaryochloris* Awaji cells and those of the 750 and 778 nm bands corresponded to a decay time of 30–35 ps at 720 nm (Table 1).

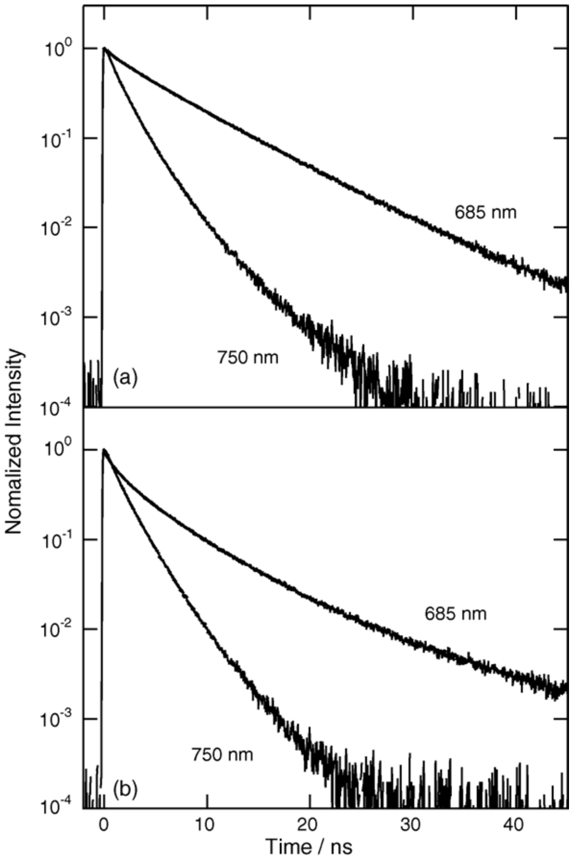

Fig. 5. Normalized fluorescence decay curves observed at −196 °C: (a) W-light grown *Acaryochloris* Awaji and (b) FR-light grown *Acaryochloris* Awaji.

In the short-wavelength region of the respective maxima, *Acaryochloris* Awaji cells showed a clear difference in their fluorescence component depending on the light-condition for growth; the W-light grown *Acaryochloris* Awaji cells exhibited the 670 nm band, whose contribution, contrarily, was very small in the FR-light grown *Acaryochloris* Awaji cells during all time regions examined (Fig. 4). This band had a relatively long lifetime whose amplitude was considerably large (7.80 ns, 63.1%), and located at a longer wavelength than that of APC. Therefore, the 670 nm band, especially at the later time stage, was not assigned to phycobiliproteins but to a biosynthetic intermediate(s) of a pigment(s) not incorporated in the energy transfer system.

4. Discussion

4.1. Energy migration and transfer

Acaryochloris Awaji showed three fluorescence bands at −196 °C in TRFS irrespective of their growth conditions, i.e. W-light or FR-light grown; those were located at 715, 731 and 749 nm, all of which were assigned to PS II, even though there was a large red-shift of the main peak. Short-wavelength components remained until at approximately 100 ps, indicating a slow energy flow from these components. In addition to these, the presence of PS I fluorescence was clearly indicated by a transient increase in the relative intensities at approximately 778 nm. Differences in TRFS by growth conditions were ascribed to the relative intensities of individual components. On the other hand, *A. marina* showed a fluorescence maximum at 729 nm in its TRFS [13]. It showed a short-wavelength component(s); however, that was not resolved as a clear peak, suggesting that the energy flow from this component was fast. These components were assigned to PS II Chl *d*. In the longer wavelength region of the maximum, a peak at 760 nm was transiently detected, and this was assigned to PS I fluorescence. Since its lifetime was short, it did not last until a late time region. This was in contrast to the TRFS of other cyanobacteria in which PS I fluorescence in 720–735 nm remains as a major component [14]. As a consequence, a simple red-shift of the maximum in a few nm was observed in the TRFS of *A. marina*. A difference in the TRFS between *Acaryochloris* Awaji and *A. marina* was clearly detected in the time-dependent behavior of the short-wavelength component(s).

In the case of *A. marina*, there was a fluorescence component assigned to PS I at 760 nm. When we resolved the rise and decay kinetics at this wavelength, we were not able to detect a rise term (data not shown). Since the absorption fraction of the long-wavelength component is small, it is natural to assume an energy transfer from a short-wavelength component(s), resulting in the presence of a rise term in its kinetics, even though the bulk of Chl is excited. Compared with this general phenomenon, the kinetics of *A. marina* PS I were peculiar. On the contrary, in the decay kinetics at 778 nm of *Acaryochloris* Awaji, a rise term was resolved for both types of cells. This rise term could be assigned to the energy transfer to PS I Chl *d*, however, we concluded that the rise term(s) in *Acaryochloris* Awaji would be assigned to energy migration in PS II Chl *d* based on the following results. In the case of *A. marina*, PS I fluorescence was detected both in steady state and time-resolved spectra, but a rise term was not resolved. In *Acaryochloris* Awaji, the PS I fluorescence component was merely detected as an increase in the relative intensities at 778 nm in its TRFS.

Energy transfer times among PS II Chl *d* of *Acaryochloris* Awaji varied depending on growth conditions; in the initial time range, the short-wavelength region of the 750 nm maximum was larger in its relative intensity in the FR-light grown cells than in the W-light grown ones. It was, therefore, found that the energy transfer in PS II occurred slower in FR-light grown cells. This was confirmed from the facts that the rise terms were detected at 750 and 778 nm for the FR-light grown cells with a longer time at 778 nm than that observed for the W-light grown cells, and that a rise time was resolved only at 750 nm in the W-light grown *Acaryochloris* Awaji cells.

The difference in the relative intensities of PS I fluorescence between *A. marina* and *Acaryochloris* Awaji did not come from the relative contents of PS I in individual cells. Whereas *A. marina* cells clearly exhibited PS I fluorescence in the steady state and the transient fluorescence spectra [13], *Acaryochloris* Awaji cells did only in TRFS (Figs. 3b and 4). It was revealed from pigment analyses that the PS I/PS II ratio of *Acaryochloris* Awaji cells was 0.89 (Murakami et al., unpublished data). This value is almost the same as reported for *A. marina* cells, 0.82–1.02 [14]. Therefore, the extremely small contribution of the PS I fluorescence in *Acaryochloris* Awaji cells would not be due to PS I content, but to the content of the Chl *d* molecules with a longer wavelength form in PS I. In the case of *Gloeobacter violaceus*, a cyanobacterium, PS I fluorescence was completely missing due to the lack of the red Chl *a* molecules [17]. The existence of PS I fluorescence in the TRFS of *Acaryochloris* Awaji ensured the existence of red Chl *d* molecules. It was found that the architectures of antenna systems in *Acaryochloris* Awaji are different from those in *A. marina*; the low-energy Chl *d* molecules of PS I are present in both *Acaryochloris* Awaji and *A. marina*, whereas those of the PS II are present only in *Acaryochloris* Awaji.

Fluorescence spectra of the two types of *Acaryochloris* Awaji at room temperature appeared differently; a contribution of the short-wavelength forms was very large for the W-light grown cells, and very limited for the FR-light grown ones. At room temperature, it is reasonable to assume thermal equilibrium among Chl molecules [18], and this is applicable to *Acaryochloris* Awaji. Therefore, fluorescence from short-wavelength forms was reasonable, however, was weak in the FR-light grown cells. This might be due to a fast energy transfer to the non-fluorescent or short-lived PS I Chl *d*, or a low content of the short-wavelength form(s) of PS II Chl *d*. Absorption spectra of the two types of cells are similar to each other (Fig. 1), suggesting that the difference in the relative content of PS II Chl *d* was not significant. Together with a slow energy migration among PS II Chl *d* in the FR-light grown cells, we are tempted to conclude that the energy flow to PS I Chl *d* is faster in the FR-light grown cells.

4.2. *Primary electron donor of PS II*

It is well known that delayed fluorescence (DF) originates from the charge recombination between the primary electron donor and the primary electron acceptor in the PS II reaction center. The lifetime of DF is longer than 10 ns; therefore, DF can be easily distinguished from other fluorescence components, because the lifetime of Chl *a* is at most 6 ns even when energy acceptors do not exist. A pigment content of *Acaryochloris* Awaji was preliminary surveyed and Chl *a* was detected as a minor component, as in the case of *A. marina* (Murakami et al., unpublished data). Therefore, DF from Chl *a* is not enigmatic in terms of pigment species.

In TRFS at the later time stage, *Acaryochloris* Awaji cells showed two peaks at 670 and 749 nm (Fig. 4). However, in the Chl *d* fluorescence region (704–778 nm), the longest lifetime was analyzed to be in the range of 5–7 ns (Table 1), suggesting that the primary electron donor of PS II would not be Chl *d*. The DF was resolved in the decay at 685 nm (11.5 ns for the W-light grown cells and 12.8 ns for the FR-light grown ones, Fig. 5 and Table 1). This long-lived component was not resolved at 670 nm, suggesting that its bandwidth is not wide. Therefore, it is reasonable to conclude that the DF was observed in the wavelength region for Chl *a* fluorescence, not in the wavelength region for Chl *d* fluorescence. This is consistent with the results for *A. marina* cells (14.0 ns, 685 nm) [13,14]. In the TRFS, clear peaks for DF of Chl *a* were not recognized even in a time range long after excitation due to high intensities from an unidentified pigment(s), however, the DF was clearly resolved in the decay curves (Fig. 5). The existence of DF, a long-lived component of 12–13 ns, indicates that the primary electron donor of PS II of *Acaryochloris* Awaji would be Chl *a*.

4.3. Diversity in the antenna system of Acaryochloris sp. in the frame of cyanobacteria

It has been thought that the fluorescence properties of oxygenic photosynthetic organisms at room temperature, that is the origin of PS II fluorescence at room temperature, are strictly conserved. The 685 nm fluorescence is observed in almost all oxygenic photosynthetic organisms in the group of cyanobacteria, red algae and Chl *a*/*b* organisms (green algae and higher plants). To date, no exception has been reported. Variation in the location of the peak is limited to ± a few nanometers. Even though we do not clearly understand the reason or mechanism of this conserved property, it is an easy assumption that the conserved organization of PS II, especially a molecular arrangement of the special pair, is one reason for this conserved property. This expands to the fluorescence spectrum at cryogenic temperature; at −196 °C, three bands are usually observed at 685, 695 and 720–735 nm with a variation of a few nanometers, with the former two assigned to PS II and the third to PS I [19]. While the 695 nm component is sometimes missing, the 685 nm component is always observed.

However, we have detected a large difference in the fluorescence properties in the genus of *Acaryochloris*. Even at room temperature, *Acaryochloris* Awaji shows a red-shifted fluorescence maximum by 14–21 nm (212–399 cm^{-1}) more than that of *A. marina*, and this red-shift (347 cm^{-1}) was also observed at −196 °C. As seen in the spectrum at −196 °C (Fig. 3), *Acaryochloris* Awaji has an extra fluorescence component (750 nm component) in addition to the band (731 nm) common to both species and will work as an energy sink; thus, the large red-shifted band is explainable. This arrangement may be analogical to the FR-fluorescence band reported in *Anacystis nidulans* (*Synechococcus* sp. PCC 6301) [20,21], though in that case, the fluorescence maximum at room temperature remained constant, not as in the case of *Acaryochloris*.

If we assume a difference in the organization of PS II that will be reflected in its fluorescence properties, *Acaryochloris* is the first example of a variation. Two species shown in this study have a small number of differences in the 16S rRNA sequence [15], but not sufficient to correspond to differentiation of species; therefore, it is surprising that the fluorescence components between *A. marina* and *Acaryochloris* Awaji show such differences.

When the fluorescence properties of Chl *a*/*c* algae (brown algae, diatom and other groups) are studied, it is easy to find differences in the room temperature fluorescence properties. In the case of the diatom, *Phaeodactylum tricornutum*, the PS II fluorescence was detected at 681 nm [22]. Even if this difference might be significant when compared with Chl *a*/*b* organisms, it is still just a small difference (86 cm^{-1}). These results indicate that we do not yet have a general understanding of PS II fluorescence, and the common fluorescence features at room temperature in the oxygenic photosynthetic organisms remain to be better clarified; surely, a topic for future studies.

5. Summary

We examined the fluorescence properties of the newly discovered Chl d-dominating cyanobacterium, *Acaryochloris* sp. strain Awaji, by comparing them with those of *A. marina* MBIC11017. As differences between two species it was found that: (1) the PS II of *Acaryochloris* Awaji cells contains Chl *d* molecules of lower excitation energy that work as an energy sink, (2) as a common characteristic, the red Chl *d* in the PS I of *Acaryochloris* Awaji has a low content and (3) the primary electron donor of PS II was Chl *a* for *Acaryochloris* Awaji cells.

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