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Fluorescence properties of chlorophyll *d*-dominating prokaryotic alga, *Acaryochloris marina*: studies using time-resolved fluorescence spectroscopy on intact cells

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Abstract

Antenna components and the primary electron donor of the photosystem (PS) II in the Chlorophyll (Chl) *d*-dominating prokaryote, *Acaryochloris marina*, were studied using time-resolved fluorescence spectroscopy in the ps time range. By selective excitation of Chl *a* or Chl *d*, differences in fluorescence properties were clearly resolved. At physiological temperature, energy transfer was confirmed by a red shift of emission maximum among PS II antenna components, and the equilibrium of energy distribution among Chl *a* and Chl *d* was established within 30 ps. A fluorescence component that can be assigned to delayed fluorescence (DF) was observed at 10 ns after the excitation; however, it was not necessarily resolved by the decay kinetics. At −196°C, a red shift of emission maximum was reproduced but the equilibrium of energy distribution was not detected. DF was resolved in the wavelength region corresponding to Chl *a* by spectra and by decay kinetics. The lifetime of the DF was estimated to be approx. 15 ns, and the peaks were located at 681 and 695 nm, significantly shorter wavelengths than those of Chl *d*. These findings strongly suggest that an origin of DF is Chl *a*, and Chl *a* is most probably the primary electron donor in the PS II reaction center (RC). These results indicate that the constitution of PS II RC in this alga is essentially identical to that of other oxygenic photosynthetic organisms.

Keywords: Chlorophyll *d*; Delayed fluorescence; Energy transfer; Photosystem II; Reaction center; *Acaryochloris marina*

1. Introduction

Acaryochloris marina is a newly discovered photosynthetic prokaryote [1] in which chlorophyll (Chl) *d* is a major antenna pigment (>95%, [2]). The Chl *a* content is variable but always a minor component. In addition to these two Chl species, *A. marina* contains phycobiliproteins and carotenoids; however, in contrast to other cyanobacteria, phycobiliproteins are not an efficient antenna [3]. The absorption maximum of Chl *d* in organic solvents is located at a wavelength longer than that of Chl *a* by about 30

Abbreviations: Chl, chlorophyll; DF, delayed fluorescence; Pheo, pheophytin; PS, photosystem; RC, reaction center; TRFS, time-resolved fluorescence spectrum

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nm. In vivo, the absorption maximum of the cells is found at approx. 715 nm, significantly longer than that of Chl *a*. According to Miyachi et al. [4], Chl *d* functions as an efficient antenna for both photosystems (PSs), thus the energy transfer to the individual reaction centers (RC) should be very efficient.

In intact cells of *A. marina*, several absorption bands of Chls were resolved by the low-temperature absorption spectra [5]: Chl *a* peaking at 670 nm, and Chl *d* peaking at 694, 714, 726 and 740 nm. Multiple chlorophyll forms in the in vivo absorption spectra, as well as in the fluorescence spectra, are common to this alga [5] as is also the case in other oxygenic photosynthetic organisms [6]. There are several fluorescence forms in *A. marina* cells at room temperature. One is a peak at 720 nm, while another located at approx. 695 nm was not clearly resolved. This peak (720 nm) is wide and of high intensity. Yet another longer wavelength emitting peak was observed at approx. 750 nm. At −196°C, the 720 nm peak was shifted to the red [5]; at 729 nm by the excitation of Chl *d* (at 461 nm) and at 730 nm by the excitation of Chl *a* (at 430 nm). A relatively high fluorescence intensity was observed around 760 nm by the excitation of Chl *a*. Other fluorescence components were not found in the short-wavelength region of the peak.

A difference in the pigment species also affects the constitution of the RC. According to Hu et al. [7], isolated PS I particles showed a flash-induced difference maximum at approx. 740 nm. This strongly indicates that the primary electron donor of RC, which corresponds to P_{700} in other oxygenic photosynthetic organisms, consists of Chl *d*, instead of Chl *a*. In contrast, the primary electron donor of RC II is still under debate. In general, it is hard to find a signal from this component. There are not many data on the PS II electron donor, unlike the case of P_{700} in PS I.

Delayed fluorescence (DF) is one of the marker signals from the primary electron donor of PS II [8]. DF is found in isolated spinach chloroplasts [9,10] and in the purified PS II RC complex (D_1-D_2-cyt b_{559}) [10,11]. Lifetimes of DF are not necessarily constant among preparations; in isolated chloroplasts, it is approx. 20 ns [12,13], and it elongates to 35 ns in the isolated PS II RC complex [10,11]. Those lifetimes are easily distinguished from other signals due to their characteristic long lifetime. Even for Chl *a* in organic solvents, the lifetime is at most 6–7 ns [14], and it is difficult to construct an artificial system with a longer lifetime than a mean lifetime of a pigment in the monomeric state. In this sense, the DF signal can be regarded as a selective index for the PS II electron donor.

We therefore measured the time-resolved fluorescence spectrum (TRFS) in the ps and ns time range of cells of the Chl *d*-dominating alga, *A. marina*, in which the organization of PS II still remains unclear. The main function of PS II is to generate a high redox potential to oxidize water, and it is generally accepted that the PS II organization was modified in the course of evolution to produce such a high potential. We found DF in *A. marina* peaking shorter than 700 nm with a lifetime of 15 ns. Such a component was not found in the wavelength region of Chl *d* emission.

2. Materials and methods

2.1. Algal culture

A. marina was grown in an oblong flask containing 500 ml of the K+ESM medium [15] under white fluorescent light (80 μE/(m^2 s), 12 h/12 h light/dark cycle) at 28°C. The medium was stirred with gentle air bubbling. Cells at the stationary growth phase were harvested and used for measurements without any treatment.

2.2. Spectroscopy

The TRFS and fluorescence lifetime were measured with the time-correlated single-photon counting apparatus described previously [16]. The light source was a Ti-Sapphire laser (Mira, Coherent, USA) and its second harmonic was generated by an LBO crystal. Fluorescence at the right angle to the excitation beam was detected by a micro-channel plate photomultiplier (R-1564-05U, Hamamatsu Photonics, Hamamatsu, Japan) after passing through an appropriate filter(s) (Toshiba, Tokyo, Japan) and a monochromometer (P-250, Nikon, Tokyo, Japan). The time zero was set to the time when an excitation pulse showed the maximum counts on a time-to-am-

plitude converter. Since an excitation pulse (pulse width of 150 fs) was observed with a width of approx. 30 ps on a time-to-amplitude converter, a negative time was assigned to a signal prior to the maximum of excitation pulse. Spectral and time resolution was 1.5 nm per channel and 2.6 ps per channel, respectively. An ambiguity of the location of the fluorescence peak was ±1 nm. A spectral sensitivity of the detector system was not corrected (see Section 3). All spectral data were transferred to a microcomputer and processed. Fluorescence lifetimes were estimated by a convolution calculation [16].

Cell density was adjusted to less than 3 μg/ml Chl. Measurements were carried out at 22°C and −196°C. For the low temperature spectroscopy, a homemade Dewar bottle and its container were used. Fluorescence from the front surface was detected. Polyethylene glycol (average molecular weight 3000) was added to a cell suspension (final concentration, 15%) to obtain homogeneous ice at −196°C.

3. Results

3.1. The excitation wavelength dependence of TRFS at physiological temperature

A. marina possesses two kinds of Chls, Chl *d* (>95%) and Chl *a*. Since the Chl *d* is a major pigment and the two Chls have different spectroscopic characteristics, it was possible to selectively excite Chl *d*; however, this was not necessarily the case for Chl *a*. Chl *a* was sensitized by excitation at 440 nm, but more than half of the light was absorbed by Chl *d*. The resulting TRFS, nevertheless, showed a clear difference (Fig. 1). The fluorescence maximum was observed at 720 nm soon after the excitation of Chl *a* at 440 nm as shown by a vertical line in Fig. 1 (left), and the peak location remained constant up to 1 ns (data not shown). The relative intensity in the short wavelength region of the peak (typically at 700 nm) was high (about 25% of the peak) at −21.1 ps and decreased to 18% in approx. 30 ps. In contrast, the relative intensity in the red region of the peak (typically at 750 nm), was approx. 13% at the beginning, and it grew to 20% of the peak after 30 ps. After this time, the time-dependent spectral change was not detected (data not shown), indicating that

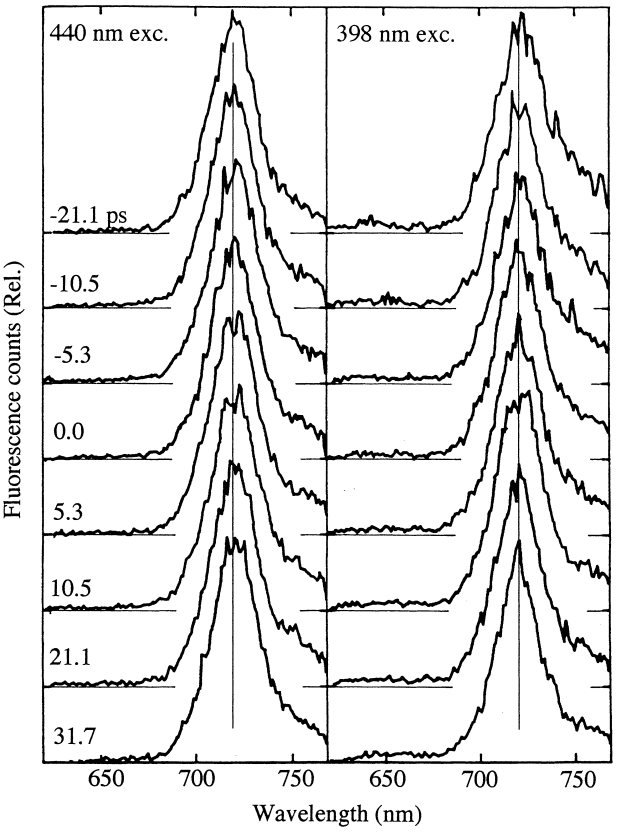

Fig. 1. TRFS of intact cells of *A. marina* at physiological temperature (22°C) in the short time range. Spectra were obtained by excitation at 440 nm (mainly Chl *a*, left), and at 398 nm (preferentially Chl *d*, right). Figures on the left stand for times after the excitation. Spectra at every 2.6 ps were recorded. A definition of a negative time is given in Section 2. Spectra were normalized to the maximum intensity of individual spectra. A vertical line shows the location of the fluorescence maximum at the longest time in the figure.

thermal equilibrium of the energy distribution was established in about 30 ps.

On the other hand, a peak was observed at 720 nm by excitation of Chl *d* at 398 nm, the same wavelength as that sensitized by Chl *a* (Fig. 1, right). A marked difference was the relative fluorescence intensity in the wavelength region longer than that of the maximum, for example at 750 nm. This emission came from Chl *d*, as judged by the locations, and the relative amplitude was approx. 35% in the initial time range, and decreased to 20% in 30 ps. At 30 ps after the excitation, however, this higher emission was no longer observed; the emission spectra sensitized by Chl *d* were superimposable to those sensitized by Chl *a*. This clearly indicates the thermal

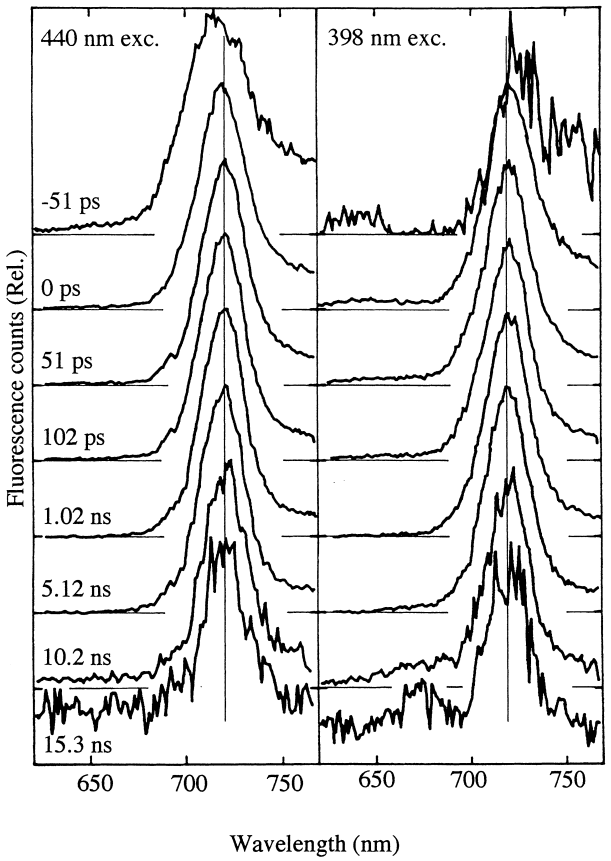

Fig. 2. TRFS of intact cells of *A. marina* at physiological temperature (22°C) in a long time range. Spectra were obtained by excitation at 440 nm (mainly Chl *a*, left), and at 398 nm (preferentially Chl *d*, right). Figures on the left stand for times after the excitation. Spectra at every 25.3 ps were recorded. A definition of a negative time is given in Section 2. Spectra were normalized to the maximum intensity of individual spectra. A vertical line shows the location of the fluorescence maximum at the longest time in the figure. Other experimental conditions were the same as shown in Fig. 1.

equilibrium of energy distribution among all antenna components. Essentially identical [17] or similar [18] equilibration processes were observed in other cases.

On a longer time scale, a difference in the TRFS due to different excitation wavelengths was observed only in the initial time range (Fig. 2). Before the maximum of the excitation pulse, which was shown as a negative time (i.e., −51 ps), the peak was observed at 715 nm by excitation at 440 nm (Chl *a*), and the bandwidth of the emission band was wide (Fig. 2, left), indicating that Chl *d* was also excited by this condition. This was reasonable because more than 95% of total Chl was Chl *d*; thus even by the excitation at 440 nm, Chl *d* absorbed a certain amount of light. A significant fluorescence intensity was observed in the wavelength shorter than the peak, i.e. from 650 to 700 nm. This indicates the presence of a component(s) in this region, and this most probably comes from Chl *a*. Upon excitation of Chl *d* (398 nm), a peak was detected at 725 nm and a relative intensity in the wavelength region longer than the peak was also high (Fig. 2, right). The peak wavelength was shifted to the blue in a short time, and reached 720 nm, the same wavelength as that excited at 440 nm. In the time range from 0 ps to 5 ns, there is essentially no difference. This was a good index of the thermal equilibration of the energy migration among antenna components. At 15 ns after the excitation, the relative intensity in the wavelength region from 650 to 700 nm increased, indicating an appearance of a new band(s). This probably corresponded to DF from the PS II (see later).

3.2. *Decay kinetics of fluorescence components at room temperature*

Decay kinetics of fluorescence detected at several wavelengths are shown in Fig. 3. Differences in the decay kinetics due to a difference in the excitation wavelength were negligible, thus decay curves sensitized by Chl *d* are shown in the figure. A time resolution in this analysis was 25.3 ps per channel, thus a very short-lived component could not be resolved, even if present. The main fluorescence lifetime was approx. 2 ns in the decays at several wavelengths from 695 to 760 nm (Table 1). In addition, there were two components whose lifetimes showed a clear

Table 1
Fluorescence lifetimes of *A. marina* at physiological temperature (22°C)

Wavelength (nm)	τ_1 (ps)	A_1	τ_2 (ps)	A_2	τ_3 (ps)	A_3
695	64	0.539	901	0.230	2154	0.231
715	86	0.440	883	0.251	2031	0.309
725	46	0.566	822	0.196	1993	0.238
740	25	0.792	709	0.090	1941	0.117
750	24	0.839	621	0.071	1937	0.090
760	33	0.796	631	0.091	1951	0.113

These lifetimes were obtained by the excitation of Chl *d* at 398 nm. A stands for a normalized amplitude of individual components.

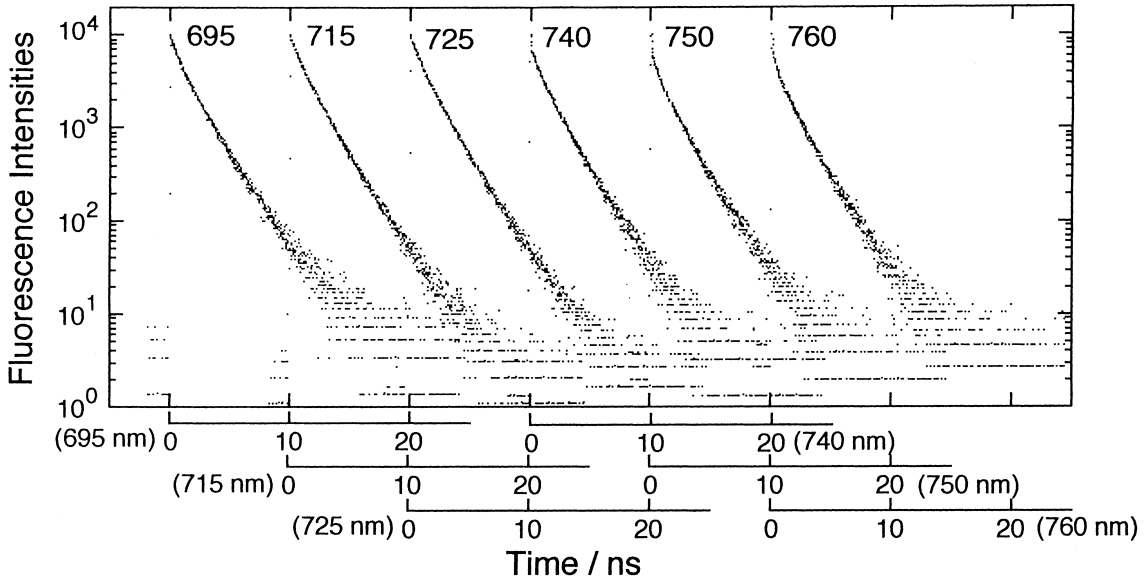

Fig. 3. Fluorescence decay profiles of intact cells of *A. marina* at 22°C. Decay kinetics were measured under excitation at 398 nm. The time resolution in these analyses was 25.3 ps per channel. The maximum fluorescence count was adjusted to 10 000 in each measurement. Figures above decay curves stand for wavelengths of decay measurements. Fluorescence lifetimes were estimated by a convolution calculation and shown in Table 1.

wavelength dependence. At a wavelength shorter than 725 nm, two components with lifetimes of 890 ps and approx. 75 ps were detected. On the other hand, in the wavelength region longer than 740 nm, a short-lived component was clearly observed in the initial phase of the decay curve and its lifetime was resolved to be 28 ps. In addition, a 625 ps lifetime component was also detected. Presence of a very short-lived component in the wavelength region longer than the emission maximum was reported on PS I particles from spinach chloroplasts [19] and in cells of *Chlorella pyrenoidosa* [20], and this was assigned to PS I emission at room (or physiological) temperature [12] (35 ps for PS I particles and 100 ps for *Chlorella* cells), even though it was not resolved by the TRFS. It is reasonable to adopt this assignment in the case of *A. marina* based on the lifetime and fluorescence wavelength. Thus, the main fluorescence at 720 nm most probably originated from PS II Chl *d*. In this aspect, fluorescence properties of antennae were essentially identical to those of other organisms except for the red shift of the emission, which was a consequence of the replacement of the main pigment with Chl *d*. Yet, a long-lived component corresponding to DF was not resolved (Table 1).

3.3. The TRFS at −196°C

At −196°C, the main emission was observed from a red-shifted component at 726 nm by the excitation of both Chl *d* (398 nm) and Chl *a* (440 nm) (Fig. 4). In addition, there was a clear difference in the spectra by a selective excitation. By Chl *a* excitation, the bandwidth at the initial time range was wide, suggesting the presence of plural components, and a characteristic band around 765 nm was observed (Fig. 4, left). The bandwidth of the main band became narrow with time, and shifted to the red up to 730 nm, which is consistent with the peak at the steady state [5]. This suggests that TRFS did not suffer from a distortion by the spectral sensitivity of our apparatus. After 51 ps of excitation, a new band appeared around 756 nm and increased with time. Based on the location, it could be assigned to PS I emission. By Chl *d* excitation (at 398 nm), succession of fluorescence spectra was essentially identical to that sensitized by Chl *a* excitation (Fig. 4, right). A feature of the emission was the presence of the band at 785 nm only in the initial time region of the excitation. It disappeared within 20 ps and did not reappear. The main emission was located at 728

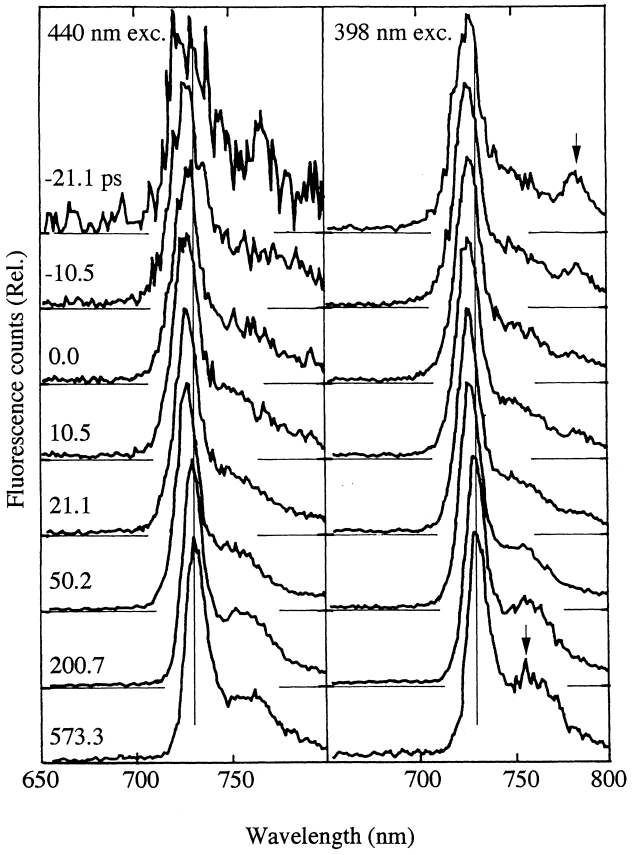

Fig. 4. TRFS of intact cells of *A. marina* at −196°C in the short time range. Spectra were obtained by excitation at 440 nm (mainly Chl *a*, left), and at 398 nm (preferentially Chl *d*, right). Figures on the left stand for times after the excitation. Spectra at every 2.6 ps were recorded. Spectra were normalized to the maximum intensity of individual spectra. A vertical line shows the location of the fluorescence maximum at the longest time in the figure. Arrows indicate fluorescence components specific to PS I. Other experimental conditions were the same as shown in Fig. 1.

nm, thus it was very hard to assign this long-wavelength component to a vibrational band of the main emission, because the maximum was located at the same wavelength as that by Chl *a* excitation. Because this band has never been reported by the steady-state spectrum, its origin was not clear at this experimental stage. Band narrowing and red shift of the emission maximum clearly indicate a heterogeneity of the emission band; the main fluorescence at 730 nm might originate from the PS II component (Chl *d*). The PS I emission appeared around 756 nm, as shown in Fig. 4. It was promoted by Chl *d* excitation, as was expected from the pigment composition of PS I. This band, however, was different from the band around 785 nm; thus the origin of the 785 nm band sensitized by Chl *d* excitation is still unclear. In TRFS in a long time scale (Fig. 5), the initial emission component sensitized by Chl *a* was a red-shifted one (Fig. 5, left), in contrast to the Chl *d* excitation (Fig. 5, right). This observation is consistent with the steady-state spectrum [5]. A probable interpretation of this difference was that a part of Chl *a* is coupled with Chl *d* whose energy level is lower than that of bulk Chl *d* in PS II. Under the low temperature condition, an equilibrium of energy migration was not the case, contrary to the observations at 22°C.

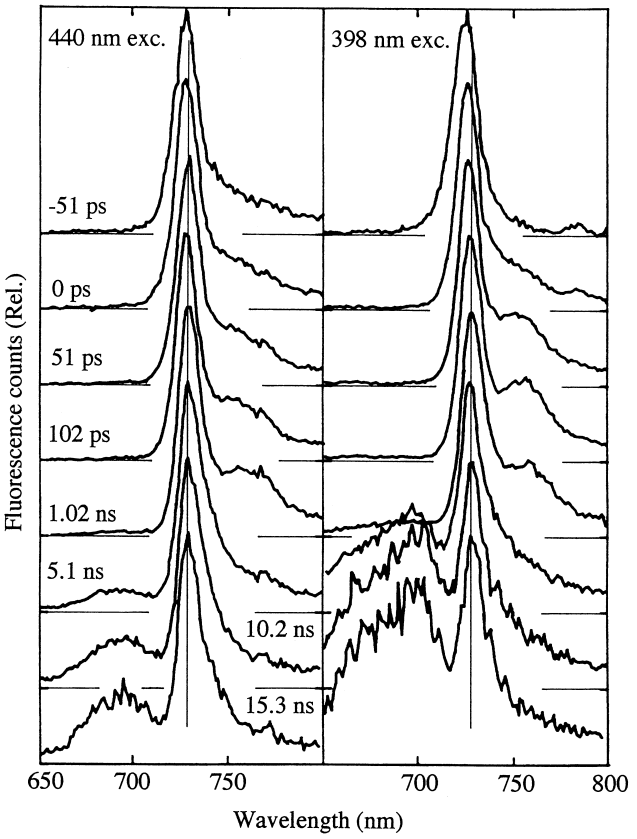

Fig. 5. TRFS of intact cells of *A. marina* at −196°C in the long time range. Spectra were obtained by excitation at 440 nm (mainly Chl *a*, left), and at 398 nm (preferentially Chl *d*, right). Figures on the left stand for times after the excitation. Spectra at every 25.3 ps were recorded. Spectra were normalized to the maximum intensity of individual spectra. A vertical line shows the location of the fluorescence maximum at the longest time in the figure. Other experimental conditions were the same as shown in Fig. 1.

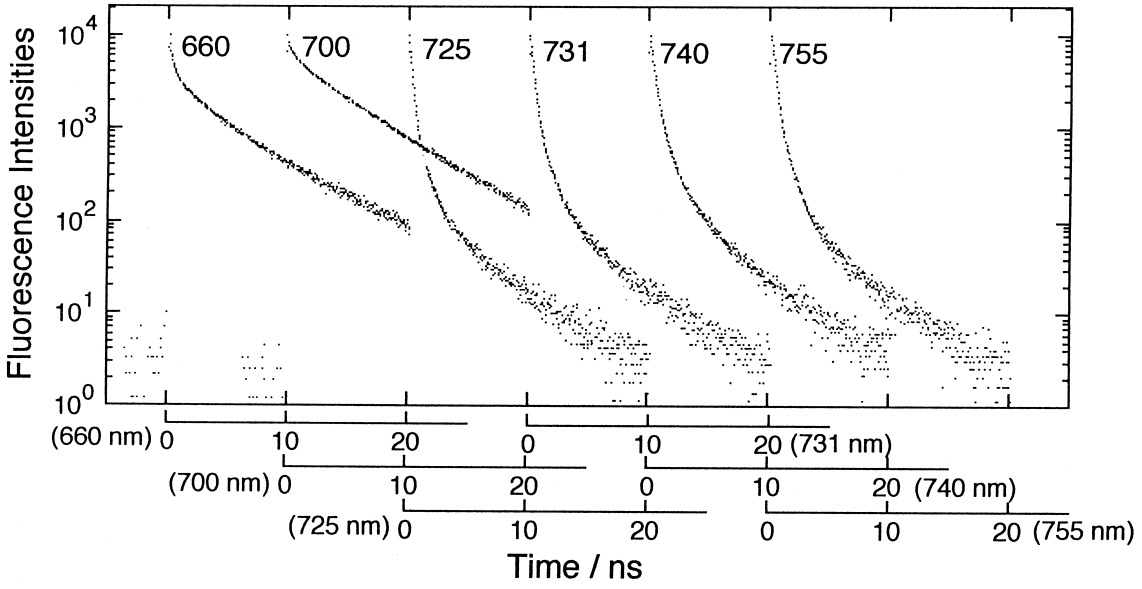

Fig. 6. Fluorescence decay profiles of intact cells of *A. marina* at −196°C. Decay kinetics were measured under excitation at 398 nm. The time resolution in these analyses was 50.6 ps per channel. The maximum fluorescence count was adjusted to 10 000 in each measurement. Numbers above decay curves stand for wavelengths of decay measurements.

3.4. Identification of DF by spectra and lifetimes

The TRFS of *A. marina* in a long time range showed the presence of a long-lived fluorescence component(s) in the wavelength region from 650 to 710 nm (Fig. 5), especially in the spectra after 5 ns of the excitation. This was also confirmed by the fluorescence decay curves (Fig. 6); a long-lived component was detected at 660 and 700 nm, and a proportion of the long-lived component was higher in the decay at 700 nm. This long-lived component, however, was not necessarily observed in the decay at wavelengths longer than 725 nm. A lifetime of this long-lived component was estimated to be 15 ns both at 660 and 700 nm, and this was assigned to DF. Its lifetime was nearly equal to that found in spinach chloroplasts (20 ns) [12,13]. In the spectra, the peak of DF was clearly observed at 695 nm, and an additional band was also assumed in the wavelength shorter than the peak. A relative intensity of the short wavelength region (typically at 681 nm) to the 695 nm band varied with time under the Chl *d* excitation condition; the ratio was smaller in a short time region (0.54 at 5.1 ns and 0.82 at 15.3 ns). In contrast, the ratio was almost constant under the Chl *a* excitation condition. This variation suggests that a significant amount of prompt fluorescence was still included in the spectra sensitized by Chl *d*, because the relative intensity of DF is determined by the energy gap and relative contents of the component pigments [16]. Thus, TRFS under the Chl *a* excitation condition showed the correct DF spectra in the wavelength region between 650 and 710 nm. The location of the second peak was estimated to be at 681 nm with the relative intensity to the 695 nm band of 0.78 ± 0.04. The main emission at 730 nm in the TRFS might contain DF; even in that case, however, the fraction of DF must be very small as shown by the decay at 731 nm (Fig. 6).

The 15 ns lifetime component was not expected from pheophytin (Pheo) *a* nor free Chl *a*, because its lifetime was reported to be 6–7 ns in organic solvents [14]. To our knowledge, there is no previous report on the lifetime of Chl *d* in organic solvents; however, it could be 15 ns only when the extinction coefficient of Chl *d* is 2.5 times smaller than that of Chl *a*, which does not seem the case for Chl *d*. Thus, the 681 and 695 nm bands were assigned to the DF originating from Chl *a*. Among these two, the 681 nm component was assigned to be the real origin of the DF; the observed intensity ratio at −196°C is explained only under the assumption that the relative

content of the 695 nm component is 300 times larger than that of the 681 nm component. This was not the case in intact cells.

4. Discussion

4.1. Origins of fluorescence and energy migration in PSs

In *A. marina*, Chl *d* is a dominant pigment and distributes to both PSs as revealed by the fluorescence spectra at physiological temperature (Figs. 1 and 2, also see [4]). The main emission at 720 nm was heterogeneous, thus a shift of the maximum was clearly resolved by the TRFS (Fig. 2). An important point was the presence of minor components in both sides of the peak wavelength. In the short wavelength region, an emission sensitized by Chl *a* was clear in the initial stage, and this was also the case for the long wavelength region, that is, around 760 nm by the Chl *d* excitation. The latter most probably came from PS I Chl *d*, as was observed in spinach chloroplasts and algae in the Chl *a*/*b* pigment system. The PS I emission at physiological temperature was confirmed by a very short-lived component in the decay kinetics (Fig. 3). The main peak of PS II shifted from 720 nm at 22°C to 730 nm at −196°C. The magnitude of the shift was about 190 cm^{-1}, and this value was similar to that observed in other oxygenic photosynthetic organisms (210 cm^{-1}, 685 vs. 695 nm), in which two different molecular species are responsible for individual emissions. Because there was no indication of the plural PS II emission bands in *A. marina* at −196°C, a difference in the PS II emission suggests the difference in the molecular organization in this alga. At −196°C, the PS I fluorescence was observed at 756 nm. A magnitude of a red shift from PS II emission at physiological temperature (720 nm) was about 660 cm^{-1}, about two-thirds of that for spinach chloroplasts (990 cm^{-1}, 685 vs. 735 nm). This suggests a difference in the depth of the trap in the whole antenna system. The PS I emission at 756 nm was short-lived (the lifetime of the major decay component was 0.9 ns); it was no longer a major peak at times longer than 3 ns after the excitation (Fig. 5). Compared with the 4.5 ns PS II emission at −196°C, it disappeared in a short time after the excitation. In the case of spinach chloroplasts, PS I emission whose lifetime is in a range of 2–3 ns was dominant up to 10 ns, and disappeared around 20 ns when the DF from PS II became dominant [12]. The short-lived PS I emission in *A. marina* is a feature of the antenna system of this alga. This short lifetime might be due to a fast energy transfer within the PS I antenna system. A similar TRFS was observed in cells of dinoflagellate, *Alexandorium tamarense* (formerly called *Protogonyaulux tamarensis*); the PS I emission has never been a dominant emission component in the spectrum [21]. To our knowledge, these two examples were rather exceptions among the oxygenic photosynthetic organisms so far studied. In this sense, the fluorescence properties of *A. marina* were different in some aspects from those observed in other algal systems.

In the case of *Anacystis nidulans* (now called *Synechococcus* sp. PCC 6301) [22,23] and *Anabaena variabilis* [23], an uphill energy transfer was reported. At room temperature, an emission of phycobiliproteins was observed by sensitization of Chl *a*. A magnitude of the energy difference between Chl *a* and allophycocyanin was approx. 550 cm^{-1}. When the same magnitude was applied to the 681 nm DF band (see below), a 708 nm antenna pigment can sensitize it. This indicates that an uphill energy transfer from Chl *d* to Chl *a* in *A. marina* is realistic.

4.2. DF as an indicator of PS II RC

The absorption maximum of Chl *d* in diethyl ether is located at 688 nm, thus this wavelength can be the shortest absorption maximum in vivo. When the Stokes shift and bandwidth are taken into account, the expected shortest fluorescence maximum is located around 695 nm in diethyl ether. The DF was resolved at 681 and 695 nm by the spectrum (Fig. 5) and by the decay kinetics (Fig. 6), and the former was assigned to the real origin based on the pigment content. The fluorescence excitation spectrum of intact cells at −196°C showed that Chl *a* contributed to the 681 nm emission (Mimuro et al., unpublished). Based on the above observations, the primary electron donor of PS II is most probably assigned to Chl *a*, common to other oxygenic photosynthetic organisms (cf. [24]). Even if the observed DF did not come from the primary electron donor of PS II, Chl *a*

energetically coupled with the primary electron donor is an origin of DF, which is probable for intact cells. The Chl *a*/Pheo *a* ratio in this alga was estimated to be nearly equal to 2 under several growth conditions (M. Kobayashi et al., personal communication), similar to the purple bacterial RC complexes [25]. These findings strongly suggest that the PS II RC complex in *A. marina* consists of four Chl *a* molecules; two Pheo *a* molecules might also be located in the PS II RC complex. Chl *a* content in intact cells was estimated to be 3–5% [2]. If Chl distribution in *A. marina* is the same as that of cyanobacteria, PS I consists of about 130 Chl [26] and PS II, about 40 Chl [27]; this was close to the reported value, 1 P_{740} per 180 Chl *d* [7]. This leads to the Chl *a* content as minimum 2.5% of total Chl in this alga. This finding was different from the constitution of PS I where Chl *d* was replaced with Chl *a* for the primary electron donor [7]. Chl *a* most probably constitutes a PS II electron donor; a very high oxidation potential is necessary for water oxidation and replacement with Chl *d* might not be able to generate such a high potential. This point might be strengthened by the amino acid analysis of *psbA* and/or *psbD* in the near future. In the case of PS I, the midpoint potential of the primary donor was reported to be lowered by the replacement with Chl *d* [7]. This might be an unfavorable indicative for the PS II primary electron donor. Until now, we have not known of oxygenic photosynthetic organisms whose PS II is constituted with pigment(s) other than Chl *a*, even if we do not exclude the possibility that Chl *d* is the primary electron donor. The PS II could be an essential machinery for oxygenic photosynthesis, thus this machinery was not modified even after the modification of the major antenna pigment.

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References

[1] H. Miyashita, H. Ikemoto, N. Kurano, K. Adachi, M. Chihara, S. Miyachi, Nature 383 (1996) 402.
[2] H. Miyashita, K. Adachi, N. Kurano, H. Ikemoto, M. Chihara, S. Miyachi, Plant Cell Physiol. 38 (1997) 274–281.
[3] J. Marquardt, H. Senger, H. Miyashita, S. Miyachi, E. Morschel, FEBS Lett. 410 (1997) 428–432.
[4] S. Miyachi, K. Strassdat, H. Miyashita, H. Senger, Z. Naturforsch. 52c (1997) 636–638.
[5] H. Schiller, H. Senger, H. Miyashita, S. Miyachi, D. Dau, FEBS Lett. 410 (1997) 433–436.
[6] C.S. French, Proc. Natl. Acad. Sci. USA 68 (1971) 2893–2897.
[7] Q. Hu, H. Miyashita, I. Iwasaki, N. Kurano, S. Miyachi, M. Iwaki, S. Itoh, Proc. Natl. Acad. Sci. USA 95 (1998) 13319–13323.
[8] W.A. Arnold, Photosynth. Res. 27 (1991) 73–82.
[9] H.J. van Gorkom, Photosynth. Res. 6 (1985) 97–115.
[10] M. Mimuro, I. Yamazaki, S. Itoh, N. Tamai, K. Satoh, Biochim. Biophys. Acta 933 (1988) 478–486.
[11] T.A. Roelofs, M. Gilbert, V.A. Shuvalov, A.R. Holzwarth, Biochim. Biophys. Acta 1060 (1991) 237–244.
[12] M. Mimuro, in: H. Scheer, W. Schneider (Eds.), Photosynthetic Light-Harvesting Systems; Organization and Function, Walter de Gruyter and Co., Berlin, 1988, pp. 589–600.
[13] F. van Mieghem, K. Brettel, B. Hillmann, A. Kamlowski, A.W. Rutherford, E. Schlodder, Biochemistry 34 (1995) 4798–4813.
[14] S.S. Brody, E. Rabinowitch, Science 125 (1957) 555.
[15] H. Miyashita, N. Kurano, S. Miyachi, J. Mar. Biotechnol. 3 (1995) 136–139.
[16] Y. Nishimura, K. Shimada, I. Yamazaki, M. Mimuro, FEBS Lett. 329 (1993) 319–323.
[17] K. Shimada, M. Mimuro, N. Tamai, I. Yamazaki, Biochim. Biophys. Acta 975 (1989) 72–79.
[18] S. Merry, S. Kumazaki, Y. Tachibana, D.M. Joseph, G. Porter, K. Yoshihara, J.D. Durrant, D. Klug, J. Phys. Chem. 100 (1996) 10467–10478.
[19] M. Mimuro, I. Yamazaki, N. Tamai, T. Yamazaki, Y. Fujita, in: T. Kobayashi (Ed.), Primary Processes in Photobiology, Springer Verlag, Berlin, 1987, pp. 23–32.
[20] I. Yamazaki, M. Mimuro, N. Tamai, T. Yamazaki, Y. Fujita, FEBS Lett. 179 (1985) 65–68.
[21] M. Mimuro, N. Tamai, T. Ishimaru, I. Yamazaki, Biochim. Biophys. Acta 1016 (1990) 281–287.
[22] R.T. Wang, J. Myers, in: S. Miyachi, S. Katoh, Y. Fujita, K. Shibata (Eds.), Photosynthetic Organelles, Special Issue of Plant and Cell Physiology, Japanese Society of Plant Physiologists, Center for Academic Publications, Japan, Tokyo, 1977, pp. 3–7.

[23] N. Murata, in: S. Miyachi, S. Katoh, Y. Fujita, K. Shibata (Eds.), Photosynthetic Organelles, Special Issue of Plant and Cell Physiology, Japanese Society of Plant Physiologists, Center for Academic Publications, Japan, Tokyo, 1977, pp. 9–13.
[24] E.J.G. Peterman, H. van Amerongen, R. van Grondelle, J.P. Dekker, Proc. Natl. Acad. Sci. USA 95 (1998) 6128–6133.
[25] H. Michel, O. Epp, J. Deisenhofer, EMBO J. 5 (1988) 2445–2451.
[26] M. Kawamura, M. Mimuro, Y. Fujita, Plant Cell Physiol. 20 (1979) 697–705.
[27] Y. Fujita, A. Murakami, Plant Cell Physiol. 28 (1987) 1547–1553.