Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1767 (2007) 327–334
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbabio

Delayed fluorescence observed in the nanosecond time region at 77 K originates directly from the photosystem II reaction center

Mamoru Mimuro [a,b,*], Seiji Akimoto [c,1], Tatsuya Tomo [a], Makio Yokono [c], Hideaki Miyashita [a,b], Tohru Tsuchiya [a,b]

[a] Department of Technology and Ecology, Hall of Global Environmental Research, Kyoto University, Kyoto 606-8501, Japan
[b] Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan
[c] Division of Biotechnology and Macromolecular Chemistry, Graduate School of Engineering, Hokkaido University, Sapporo 060-8628, Japan

Received 15 November 2006; received in revised form 14 February 2007; accepted 15 February 2007
Available online 24 February 2007

Abstract

The excited-state dynamics of delayed fluorescence in photosystem (PS) II at 77 K were studied by time-resolved fluorescence spectroscopy and decay analysis on three samples with different antenna sizes: PS II particles and the PS II reaction center from spinach, and the PS II core complexes from *Synechocystis* sp. PCC 6803. Delayed fluorescence in the nanosecond time region originated from the 683-nm component in all three samples, even though a slight variation in lifetimes was detected from 15 to 25 ns. The relative amplitude of the delayed fluorescence was higher when the antenna size was smaller. Energy transfer from the 683-nm pigment responsible for delayed fluorescence to antenna pigment(s) at a lower energy level was not observed in any of the samples examined. This indicated that the excited state generated by charge recombination was not shared with antenna pigments under the low-temperature condition, and that delayed fluorescence originates directly from the PS II reaction center, either from chlorophyll a_{D1} or P680. Supplemental data on delayed fluorescence from spinach PS I complexes are included.

Keywords: Delayed fluorescence; Photosystem II; Reaction center complex; Time-resolved fluorescence spectroscopy

1. Introduction

Delayed fluorescence, also called delayed light emission [1,2], is a non-invasively observable character of photosystem (PS) II in oxygenic photosynthetic organisms. Delayed fluorescence is known to originate from the excited state generated by charge recombination between the special pair (P680) and the primary electron acceptor pheophytin (Phe) *a* [2–5], although the actual reaction process has not yet been resolved. Delayed fluorescence consists of multiple components with different lifetimes ranging from nanoseconds to milliseconds, and even to seconds [6–13], reflecting recombination processes in intermediary reaction states of PS II. The shortest lifetime component, estimated to be in the range of 15–60 ns [8–10,14–18], is assigned to direct recombination between $P680^+$ and Phe a^-, and this reaction occurs even at cryogenic temperatures.

Delayed fluorescence has been analyzed mainly in terms of lifetimes, and its spectral properties have not been well resolved. Some earlier studies have shown that the delayed-fluorescence spectrum at physiological temperature is essentially identical to the prompt-fluorescence spectrum. In the case of spinach chloroplasts, the maximum for delayed fluorescence is located between 683 and 685 nm, reflecting the origin of delayed fluorescence in PS II [11,12]. It is not necessarily clear whether the observed delayed fluorescence comes from the PS II reaction center (RC) or from the antennae. However, it is known that at 77 K, PS II shows two prompt-fluorescence bands, typically at 685 and 695 nm [19–22]. The former band

Abbreviations: Chl, chlorophyll; CP, chlorophyll protein; Phe, pheophytin; PhQ, phylloquinone; PS, photosystem; RC, reaction center; TRFS, time-resolved fluorescence spectrum

* Corresponding author. Department of Technology and Ecology, Hall of Global Environmental Research, Kyoto University, Kyoto 606-8501, Japan. Tel./fax: +81 75 753 6855.
E-mail address: mamo-mi@mm1.mbox.media.kyoto-u.ac.jp (M. Mimuro).
[1] Current address: Molecular Photoscience Research Center, Kobe University, Kobe 657-8501, Japan.

0005-2728/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2007.02.012

comes from 43 kDa chlorophyll protein (CP43) and the latter from CP47 [23]. In addition, prompt fluorescence from LHC II, when present, is observed at 680 nm [24] (Fig. 1). The dynamics of the excited state in delayed fluorescence, including antenna components, have not yet been resolved clearly.

When we consider the excited-state dynamics in PS II, including delayed fluorescence, we can expect the following energy-migration processes at 77 K (Fig. 1). The time for the recombination process is much longer than that for energy transfer; hence it is possible that after the excited state is generated by charge recombination, energy transfer occurs from the pigment responsible for delayed fluorescence in the RC (F683) to low-energy antenna components (F685 in CP43 and/or F695 in CP47). In this case, two patterns are possible for the delayed-fluorescence spectrum. The first possibility is that delayed fluorescence comes from an equilibrated state with a lower energy component(s), i.e., F685 and/or F695. In this case, the delayed-fluorescence spectrum would exhibit two or three bands, corresponding to the pigment responsible for delayed fluorescence (F683) and its energy acceptor(s), and should not change with time. The fluorescence band would be broad because of contributions from multiple bands. If a component with an appropriate energy level is present, further energy transfer from either F685 or F695 to LHC II may also occur. The second possibility is that delayed fluorescence occurs with simultaneous energy transfer to a lower-energy component. In this case, the relative intensity of the delayed fluorescence from a lower-energy component would increase with time (F685/F695). It is also possible that energy transfer would fail to occur even if a low-energy component is present. In this case, delayed fluorescence would arise only from the initial pigment responsible for delayed fluorescence, F683.

To investigate these possibilities, we measured the time-resolved fluorescence spectrum (TRFS) in the ns time range at 77 K for three preparations with different antenna sizes: the PS II core (CP43–CP47–D1–D2–cyt b_{559}) from *Synechocystis* sp. PCC 6803 and PS II particles, called BBY particles after Berthold, Babcock, and Yocum [25], and the PS II RC, i.e., D1–D2–cyt b_{559} complexes, from spinach. We found that the excited state generated by the charge recombination at 77 K was not shared with the antenna components, and that the delayed fluorescence did not originate from an equilibrated state with antenna pigments at lower energy levels. Supplemental data on delayed fluorescence from spinach PS I complexes are also shown.

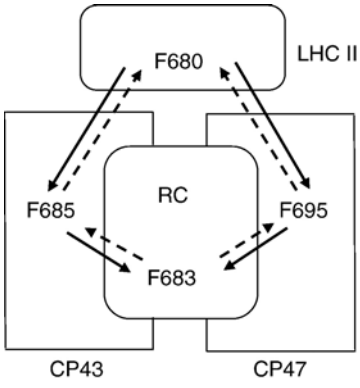

Fig. 1. Scheme for the origin of delayed fluorescence and possible energy-transfer pathways in PS II. The thick lines represent the energy-transfer pathways among prompt-fluorescence components and the broken lines represent possible energy-transfer pathways originating from delayed fluorescence in the PS II RC. Energy transfer to the PS I component is also possible (not shown).

2. Materials and methods

2.1. Sample preparation

Synechocystis sp. PCC 6803 was grown photosynthetically under previously described conditions [10]. To purify the PS II core from *Synechocystis* sp. PCC 6803, a 6×His-tag was introduced to the C-terminus of CP47 by site-directed mutagenesis [26]. Thylakoid membranes were treated with dodecyl-β-D-maltoside (0.8%) to solubilize the complexes, and the core was purified by a combination of centrifugation and Ni(II)-chelate column chromatography. PS II particles were isolated by mechanical disruption of chloroplasts, detergent treatment with Triton X-100, and differential centrifugation [25]. D1–D2–cyt b_{559} complexes were isolated from spinach by the method of Nanba and Satoh [27] with a slight modification [28]. The purity of the PS II core and D1–D2–cyt b_{559} complexes was analyzed by SDS-PAGE. Ether-treated PS I complexes were obtained by a method described previously [29].

2.2. Steady-state fluorescence spectra

Fluorescence spectra were measured with a Hitachi 850 spectrofluorometer as described previously [9]. The spectral sensitivity of the apparatus was corrected using a sub-standard lamp (Hitachi, Japan) of known radiation profile. The excitation wavelength was 435 nm for all samples.

2.3. Time-resolved fluorescence spectra

TRFS were measured by a time-correlated single-photon counting method at 77 K as described elsewhere [9,10]. The excitation wavelength was 435 nm and the repetition rate of the pulse trains was 2.9 MHz, which did not interfere with the measurements up to 50 ns (51.7 ps/channel × 1024 channels). The spectral resolution of the TRFS was within 2 nm.

Under our conditions, the excitation light was provided as a flash of a very short duration (approximately 80 fs). The time-averaged flash intensity was low and, therefore, the probability of light absorption by individual RCs was low. There was almost no possibility of double hits from one flash, and the actual repetition rate for individual RCs was significantly reduced when compared with the apparent rate (2.9 MHz). Furthermore, when detecting photons from samples, the counting rate was suppressed to less than 1% of actinic photons. These factors ensured that the fluorescence from samples was detected as a probability-based phenomenon.

3. Results

3.1. PS II particles from spinach

The antenna size of PS II particles has been reported to be between 200 and 250 chlorophylls (Chls) per 4 Mn atoms [25]. A small amount of PS I remained in the preparations. The steady-state fluorescence spectrum at 298 K with excitation at 435 nm showed a maximum at 683 nm (Fig. 2A). At 77 K, a prominent LHC II peak at 680 nm and a shoulder at 695 nm were observed (Fig. 2B). Upon excitation at 435 nm, the TRFS at 77 K showed that the main prompt-fluorescence peak was initially located at 680 nm, but its intensity soon decreased (Fig.

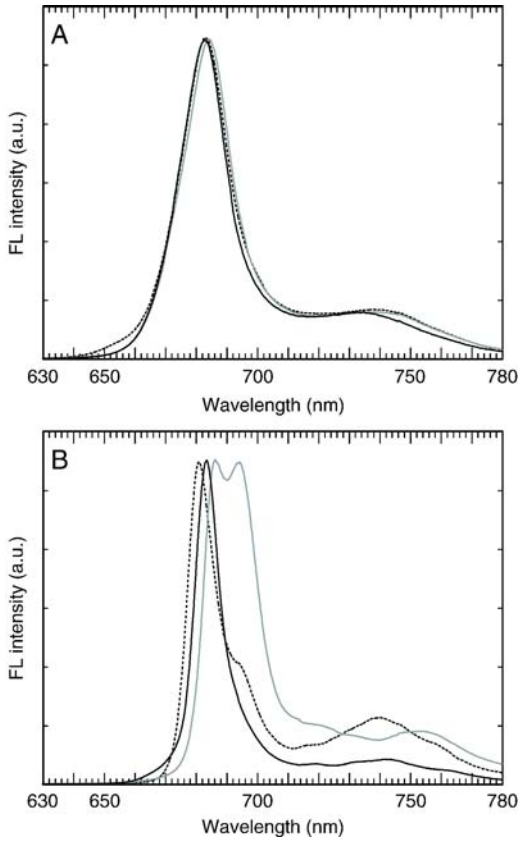

Fig. 2. Steady-state fluorescence spectra of the three PS II samples. Spectra measured at (A) 298 K and (B) 77 K. The excitation wavelength was 435 nm (bandwidth 5 nm) and fluorescence was detected (bandwidth 2.5 nm) for PS II particles (dotted black line), the PS II RC (solid black line), and the PS II core from *Synechocystis* sp. PCC 6803 (solid gray line).

3). The peak shifted to the red (683 nm) after approximately 100 ps, and to 684 nm after 500 ps. At approximately 100 ps, a third component was detected at 695 nm and a fourth at 732 nm. This last component showed a red shift to 735 nm over time. These four prompt-fluorescence bands were assigned to LHC II, PS II, PS II, and PS I, respectively. This result is typical time-dependent behavior for the Chl *a*/*b* system.

Over the longer time range, PS I prompt fluorescence was no longer detected at 20 ns due to its short lifetime (3.5 ns), whereas the two PS II components located at 684 and 695 nm were discernible for up to 48 ns. The relative intensity of the 684-nm component increased with time. This indicates that the origin of delayed fluorescence is the 684-nm component and that equilibrium is not established between the two components. The absence of a long-lived PS I fluorescence further indicates that energy transfer from the 684-nm delayed-fluorescence component to the PS I antenna did not occur. The lifetime of delayed fluorescence was estimated to be 21.8 ns (amplitude 0.1%) based on fluorescence decay curves at 683 nm. This was consistent with our estimates for spinach chloroplasts (20.7 ns). The lifetimes and amplitudes of the other components at 683 nm were 118 ps (63.4%), 423 ps (27.0%), 1.41 ns (8.3%), and 4.51 ns (1.2%). All of these were assigned to prompt fluorescence. The amplitude of delayed fluorescence was very small, such that even long after excitation, the TRFS did not necessarily show the bands assigned to delayed fluorescence and a portion of it was assigned to prompt fluorescence.

3.2. PS II core of Synechocystis sp. PCC 6803

This preparation had an antenna size of approximately 40 Chls [30,31], close to that found in the crystal structure of *Thermosynechococcus elongatus* PS II [32]. The steady-state fluorescence spectrum showed a maximum at 683 nm at 298 K. At 77 K, two prominent peaks were observed at 686 and 695 nm (Fig. 2B). The TRFS at 77 K showed that in the initial time period the main prompt fluorescence was observed at 682 nm with a shoulder at 677 nm (Fig. 4, solid lines). The main peak gradually shifted to 685 nm after approximately 500 ps. The 694-nm component appeared at approximately 300 ps after excitation, but did not disappear up to 48 ns. The relative intensity of the 685-nm component was lower from 1.8 to 22 ns, but became higher after 43 ns. This was the same behavior as observed in the PS II particles (Fig. 3). The lifetime of delayed

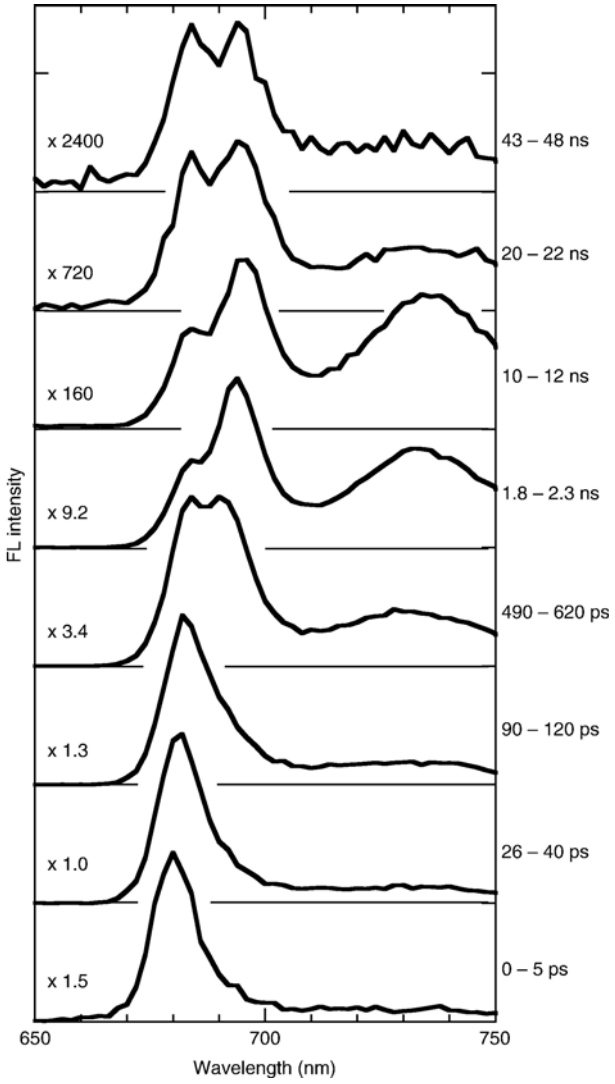

Fig. 3. TRFS at 77 K for PS II particles isolated from spinach chloroplasts. The excitation wavelength was 435 nm. Numbers shown on individual spectra indicate magnification factors relative to that of the most intense spectrum.

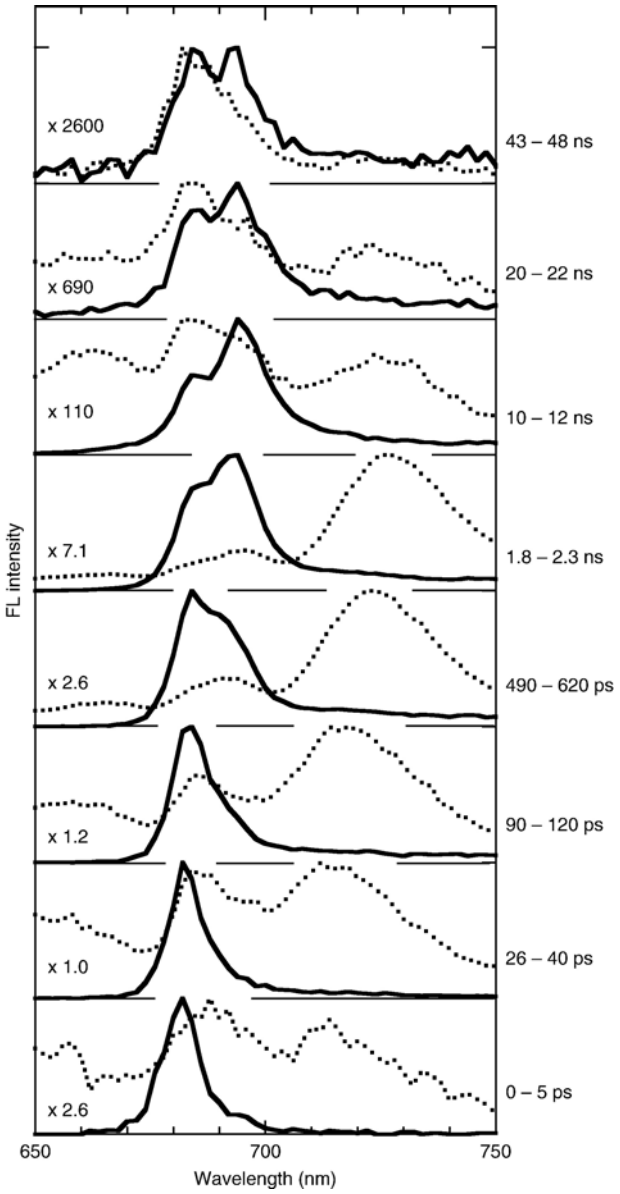

Fig. 4. TRFS at 77 K for the PS II core of *Synechocystis* sp. PCC 6803. Measurement conditions were as for Fig. 3. The dotted line shows the spectrum for intact cells of *Synechocystis* sp. PCC 6803 [10].

fluorescence in this preparation was 21.5 ns (amplitude 0.1%), which was significantly longer than the 15.4 ns observed in intact cells [10]. The lifetimes and amplitudes of other prompt-fluorescence components at 683 nm were 120 ps (57.5%), 527 ps (26.8%), 1.55 ns (13.2%), and 4.50 ns (2.4%).

Intact cells of *Synechocystis* sp. PCC 6803 showed a different TRFS than that observed in the PS II core (Fig. 4, dotted lines). The PS I fluorescence observed at 727 nm disappeared almost completely within 12 ns, reflecting the short lifetime and the absence of energy transfer from the 683-nm component responsible for delayed fluorescence. The PS II fluorescence observed at 695 nm also disappeared within approximately 22 ns after excitation. A single component was observed at 683 nm at 43 ns after excitation, indicating that this band was responsible for delayed fluorescence. The peak was observed at a slightly shorter wavelength than the fluorescence peak of the PS II core, although the difference was small.

3.3. PS II RC (D1–D2–cyt b_{559}) from spinach

This preparation contained 6 Chl *a* molecules per 2 Phe *a* molecules, and more than 90% was in dimeric form [28]. It was more stable than the preparation obtained by Nanba and Satoh [27], as indicated by a red-shifted absorption maximum at 676 nm (data not shown). The steady-state fluorescence spectrum showed a single maximum at 683 nm at both 298 K and 77 K (Fig. 2B). The time-dependent behavior of fluorescence in the PS II RC at 77 K (Fig. 5) was different from that of the PS II particles (Fig. 3) or the PS II core from *Synechocystis* sp. PCC 6803 (Fig. 4). In the initial time period, the main fluorescence band was rather broad, consistent with contributions from the 678- and 682-nm bands. After several hundred ps, the first band decreased, leaving the main band at 682 nm. The second band at 692 nm appeared at approximately

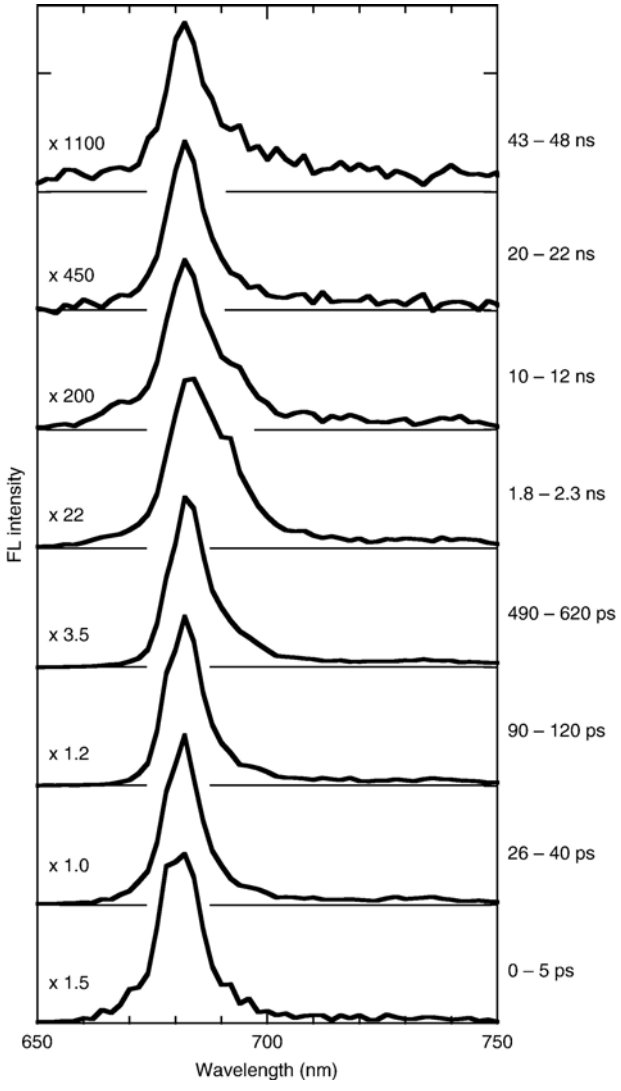

Fig. 5. TRFS at 77 K for D1–D2–cyt b_{559} isolated from spinach chloroplasts. Measurement conditions were as for Fig. 3.

1 ns after excitation, but had almost disappeared by 10 ns, due to its short lifetime. The latter component was not observed in a previous study [8], and may indicate a new state resulting from the presence of a dimer. After 10 ns, only the 682-nm component was observed. This indicates that the time-dependent behavior of the 692-nm component was distinct from that of the 682-nm component and that no equilibrium was achieved between the two components. The delayed-fluorescence lifetime in this preparation was 25.3 ns (amplitude 0.2%). This lifetime was a little longer than that observed for the BBY particles (21.8 ns), but significantly shorter than in our previous study on the monomer of D1–D2–cyt b_{559} (39.8 ns) at 77 K [8]. The lifetimes and amplitudes of the other components at 682 nm were 45 ps (57.1%), 312 ps (32.3%), 927 ps (9.5%), and 4.36 ns (0.9%). When we compared the fluorescence bands observed at long times after excitation, we noticed that the PS II RC showed a band at a shorter wavelength (682 nm) than that observed for spinach PS II particles (Fig. 3) and the PS II core of *Synechocystis* sp. PCC 6803 (Fig. 4).

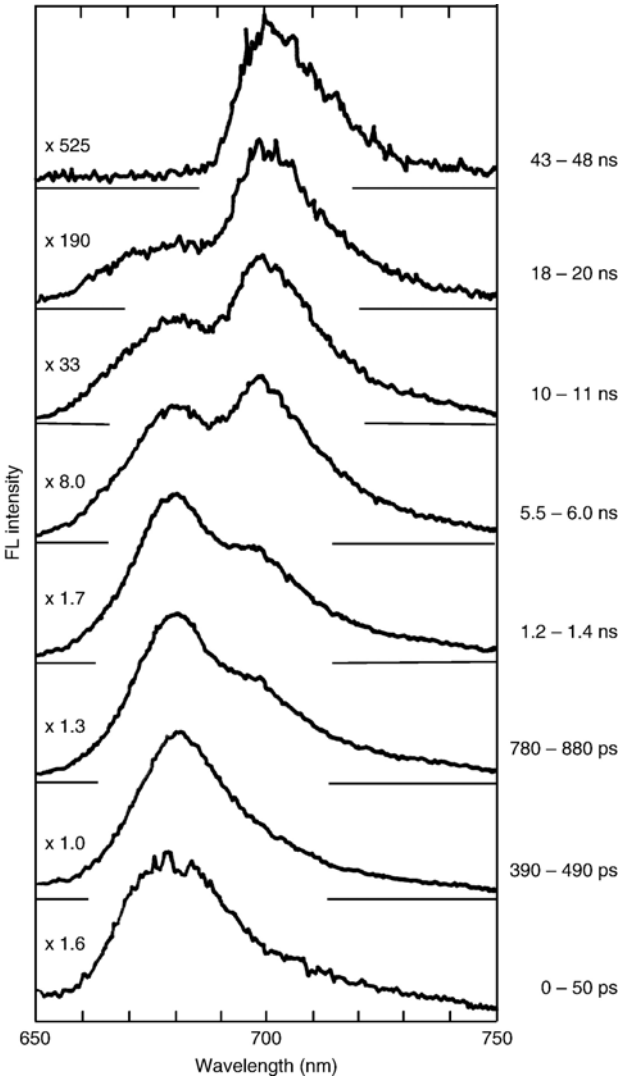

Fig. 6. TRFS at 77 K for ether-treated PS I complexes isolated from spinach. Measurement conditions were as for Fig. 3. The interval of data collection was approximately 1 nm.

Delayed-fluorescence yields were estimated by multiplying lifetimes and amplitudes and the yields are closely related to the steady-state fluorescence intensities. The yield was 7.3% for spinach PS II particles and increased to 14.8% for the spinach PS II RC. Higher yields in samples with smaller antenna sizes were clearly observed in our measurements. The yield in *Synechocystis* sp. PCC 6803 was lower than in spinach, but the reason for this is not clear at present.

3.4. Ether-treated PS I complexes of spinach

In general, delayed fluorescence is not detected in PS I because charge recombination does not occur, due to fast electron transfer from the primary electron acceptor (A_0) to the secondary electron acceptor phylloquinone (PhQ). Delayed fluorescence is observed only in the absence of PhQ. It has been reported that treatment with ether can extract PhQ from PS I complexes [33] and induce delayed fluorescence [33,34]. We measured the TRFS at 77 K on ether-treated PS I complexes of spinach containing 13 Chl molecules. The prompt-fluorescence band with a maximum at 678 nm completely disappeared 43 ns after excitation, and delayed fluorescence was observed at 701 nm (Fig. 6), originating from direct recombination between $P700^+$ and A_0^-. The most probable origin of the delayed fluorescence was the excited state of P700 itself. The lifetime of this delayed fluorescence was estimated to be 57.4 ns, much longer than that observed for PS II samples in this study. The band shape of delayed fluorescence in the longer wavelength region was monotonous and invariant with time, indicating that energy transfer to the long wavelength component probably did not occur.

4. Discussion

Three prompt-fluorescence components are known in PS II at 77 K: the 680-nm component from LHC II [24], the 685-nm component from CP43 [23], and the 695-nm component from CP47 [23]. In addition to these, the 683-nm band is observed in isolated PS II RCs [8]. It is often not easy to distinguish the 683-nm RC band and the 685-nm CP43 band. Steady-state spectra of samples used in this study revealed differences between these two bands (Fig. 2), and the TRFS studies also showed a difference in the maxima. Therefore, we have been able to discriminate the fluorescence bands of antennae and RCs by their peak locations in the TRFS at long times after excitation: 682–683 nm for the PS II RC and intact cells of *Synechocystis* sp. PCC 6803, and 684–685 nm for spinach PS II particles and the PS II of *Synechocystis* sp. PCC 6803. Although the difference is small, it is significant.

The delayed-fluorescence spectrum could not be resolved by the analysis of decay-associated spectra, because the relative amplitude of delayed fluorescence was very small. Decay-associated spectra always give component spectra with relatively large amplitudes. Therefore, delayed fluorescence is resolved only by decay-curve analysis, as adopted in this study. However, the small amplitude of delayed fluorescence

sometimes leads to confusion in the interpretation of fluorescence bands, due to an overlap with prompt fluorescence.

Changes in the relative delayed-fluorescence intensity between the 684-nm and the 695-nm components over a long time period seemed to depend on the antenna size. PS II particles and the PS II core of *Synechocystis* sp. PCC 6803 showed a similar pattern, i.e. the relative intensity of the two was nearly equal at a late time period. However, this was not the case for the PS II RC. Therefore, one might be tempted to conclude that when the antenna size is large, delayed fluorescence arises from an equilibrium state between the pigment responsible for delayed fluorescence and the 695-nm antenna component, following energy transfer to the latter. However, the TRFS of intact cells of *Synechocystis* sp. PCC 6803 clearly shows a predominant peak at 683 nm at 43 ns after excitation (Fig. 4, dotted line), indicating that delayed fluorescence did not originate from an equilibrated state, even though a long-lasting intensity at 695 nm was observed due to an overlap with the 683-nm component. The PS II RC dimer showed a fluorescence peak at 692 nm in the TRFS, as a reflection of its short lifetime. Therefore, we conclude that the origin of delayed fluorescence is the 683-nm component from the PS II RC, and that it does not equilibrate with the 685- and 695-nm components under low-temperature conditions. Energy transfer from the 683-nm component did not occur in any of the four preparations. The 685-nm component from CP43 and the 695-nm component from CP47 overlapped the 683-nm delayed fluorescence, which made the TRFS complicated due to the large amplitudes of the former peaks. The 695-nm component was not present at later times in intact cells of *Synechocystis* sp. PCC 6803. This is explained by the fast decay of the 695-nm prompt fluorescence component. The absence of delayed fluorescence in the PS I Chl *a* region (Fig. 3, dotted lines in Fig. 4) clearly indicates that the excited state is not shared with the pigment pool that supplies excitation energy to PS I and II.

There remain a few unanswered questions regarding delayed fluorescence: (1) the site of charge recombination; (2) the assignment of the pigment responsible for delayed fluorescence; (3) the causes of differences in lifetimes; and (4) the reason for the absence of energy transfer from the pigment responsible for delayed fluorescence to the surrounding antenna component(s). These questions are closely related to the constitution and reaction processes of PS II.

The site of charge recombination has not yet been identified. Although charge separation takes place either from the special pair or from accessory Chl a_{D1} [35,36], a positive charge is localized on the special pair whenever charge recombination takes place. There are two possible sites for the charge recombination. One is on Chl a_{D1} by electron transfer from Phe *a* and hole transfer from the special pair. The other is on the special pair after two-step electron transfer from Phe *a* through Chl a_{D1} or direct transfer from Phe *a*. It has been reported that Chl a_{D1} accumulates the triplet state that is formed by recombination [37], leading to the idea that charge recombination on Chl a_{D1} is more likely. However, this does not necessarily define the recombination site, since the triplet state is distributed among pigments depending on their energy levels. The excited state formed by charge recombination does not necessarily remain on the pigment responsible for delayed fluorescence. Thus, the site of the recombination remains controversial.

There is no convincing evidence that the 685-nm prompt-fluorescence band is identical to the 683-nm delayed-fluorescence band. We have measured both prompt fluorescence and delayed fluorescence at 77 K on the monomer-type PS II RC, and have found that, of the three fluorescence components (670, 676, and 683 nm), the 683-nm fluorescence band most clearly reflects the charge-separation and charge-recombination processes [8]. This demonstrates that the 683-nm delayed-fluorescence band observed at 77 K corresponds to the prompt-fluorescence band. Since the 683-nm band is associated with the PS II RC (Figs. 2, 5) [8] and the 685-nm fluorescence band is attributed to CP43 [20,22,23], the delayed-fluorescence component is assigned to the pigment in the RC, i.e., the accessory Chl *a* (Chl a_{D1}) or the special pair (P680).

At physiological temperatures, however, another 683-nm delayed-fluorescence component might be present in the antenna. It is known that at physiological temperatures, delayed fluorescence with a long lifetime is affected by inhibitors of electron transfer and uncouplers of the energized state of thylakoid membranes [3–5,11,12]. The lifetime has been estimated to be in the microsecond to millisecond range, or even in the second range [11–13]. In this case, it is reasonable to assume that delayed fluorescence originates from a thermally equilibrated state with antenna components or from an excited state generated by other mechanisms [3–5,11]. Thus, at physiological temperature, delayed fluorescence involves processes in addition to the charge-recombination process between P680 and Phe *a*.

Theoretical investigations have demonstrated that the charge-recombination process depends on the difference in the standard free energy [12,16,17], as in the case of thermoluminescence [38–40]. The observed lifetimes for delayed fluorescence were 21.8 ns for PS II particles, 21.4 ns for the PS II core of *Synechocystis* sp. PCC 6803, 15.3 ns for intact *Synechocystis* sp. PCC 6803 cells, and 25.3 ns for the PS II RC from spinach. The lifetime was much longer (57.4 ns) with the ether-treated PS I complexes from spinach than observed for the four types of PS II preparations. The charge-recombination process is, in general, governed by the difference in the standard free energy of the intermediary states participating in the recombination [12]. The difference in free energy between the excited state of the special pair and the primary electron acceptor is smaller in PS I than in PS II [41]. The observed lifetime did not reflect this property, and the difference in the observed lifetime was significant, although the ether treatment may have induced an artifact in the system. The lifetime of delayed fluorescence is affected by the gap in free energy, but this does not appear to be the only factor. The intactness of the samples may also affect the observed lifetime, although this would be limited to the delayed fluorescence at 77 K. When we discuss the standard free energy of the intermediary components based on the delayed fluorescence

lifetimes, we need to pay attention to the intactness of samples, which is not always easy to determine.

Since the triplet state accumulates on Chl a_{D1} [37], it is likely that charge recombination also takes place on Chl a_{D1}, although this has not yet been confirmed. The energy level of Chl a_{D1} is the lowest among the eight pigments in the PS II RC. Energy transfer from Chl a_{D1}, therefore, might be suppressed when the energy gap between Chl a_{D1} and the antenna is large or when the distance between the two is long. If the energy gap between Chl a_{D1} and nearby antenna Chl is larger than the thermal energy at 77 K (approximately 50 cm^{-1}), energy transfer could be partially halted. However, the energy gap is not this large [22]. Therefore, the distance might be the more likely reason for suppression of energy equilibrium between Chl a_{D1} and nearby antenna Chl.

We have shown that in intact cells of the Chl *d*-dominated cyanobacterium *Acaryochloris marina* MBIC 11017, delayed fluorescence at 77 K is observed in the Chl *a* region with a lifetime of approximately 15 ns, but is not observed in the Chl *d* region [9,10]. It would be easy to speculate that energy transfer from Chl *a* to Chl *d* can occur, inducing delayed fluorescence in the Chl *d* region if both pigments are present. However, our observations are contrary to this, and consistent with observations on intact cells of *Synechocystis* sp. PCC 6803 [9] that delayed fluorescence originated from pigment(s) at a higher energy level. Molecular interactions among core components might be modified by heterogeneous Chl *a* and Chl *d* constituents. As shown in this study, energy transfer did not occur from the pigment responsible for delayed fluorescence to any surrounding pigments at low temperature in spinach chloroplasts or *Synechocystis* sp. PCC 6803. If this also applies to *A. marina*, it is not surprising that delayed fluorescence arises from Chl *a*, even though a convincing functional assignment for Chl *a* in the PS II RC of *A. marina* has not been established.

Acknowledgments

We thank Dr. T. Noguchi, University of Tsukuba, for his critical reading of the manuscript, and Prof. I. Ikegami, Teikyo University and Prof. I. Yamazaki, Hokkaido University, for their help with the TRFS measurements on the ether-treated PS I complexes. This study was supported by a Grant-in-Aid for Creative Scientific Research (No. 17GS0314) from the Japanese Society for the Promotion of Science and by Scientific Research on Priority Areas "Comparative Genomics" (Nos. 17018022 and 18017016) from the Ministry of Education, Sports, Culture, Science, and Technology, Japan, to MM, and by a Grant-in-Aid for Scientific Research (No. 18350067) to SA.

References

[1] B.L. Strehler, W. Arnold, Light production by green plants, J. Gen. Physiol. 34 (1951) 809–820.
[2] W.A. Arnold, Experiments, Photosynth. Res. 27 (1991) 73–82.
[3] Govindjee, P.A. Jursinic, Photosynthesis and fast changes in light emission by green plants, in: K.C. Smith (Ed.), Photochem. Photobiol. Rev., vol. 4, Plenum Press, New York, 1979, pp. 125–205.
[4] V. Goltsev, I. Zaharieva, P. Lambrev, I. Yordanov, R. Strasser, Simultaneous analysis of prompt and delayed chlorophyll *a* fluorescence in leaves during the induction period of dark to light adaptation, J. Theor. Biol. 225 (2003) 171–183.
[5] E. Tyystjärvi, I. Vass, Light emission as a probe of charge separation and recombination in the photosynthetic apparatus: relation of prompt fluorescence and delayed light emission and thermoluminescence, in: G.C. Papageorgiou, Govindjee (Eds.), Chlorophyll *a* Fluorescence: A Signature of Photosynthesis, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2004, pp. 363–388.
[6] P. Jursinic, Govindjee, C.A. Wraight, Membrane potential and microsecond to millisecond delayed light emission after a single excitation flash in isolated chloroplasts, Photochem. Photobiol. 27 (1978) 61–71.
[7] D. Wong, Govindjee, P. Jursinic, Analysis of microsecond fluorescence yield and delayed light emission changes after a single flash in pea chloroplasts: effects of mono- and divalent cations, Photochem. Photobiol. 28 (1978) 963–974.
[8] M. Mimuro, I. Yamazaki, S. Itoh, N. Tamai, K. Satoh, Dynamic fluorescence properties of D1–D2–cytochrome *b*–559 complex isolated from spinach chloroplasts: analysis by means of the time-resolved fluorescence spectra in picosecond time range, Biochim. Biophys. Acta 933 (1988) 478–486.
[9] M. Mimuro, S. Akimoto, I. Yamazaki, H. Miyashita, S. Miyachi, Fluorescence properties of chlorophyll *d*-dominating prokaryotic alga, *Acaryochloris marina*: studies using time-resolved fluorescence spectroscopy on intact cells, Biochim. Biophys. Acta 1412 (1999) 37–46.
[10] M. Mimuro, S. Akimoto, T. Gotoh, M. Yokono, M. Akiyama, T. Tsuchiya, H. Miyashita, M. Kobayashi, I. Yamazaki, Identification of the primary electron donor in PS II of the Chl *d*-dominated cyanobacterium *Acaryochloris marina*, FEBS Lett. 556 (2004) 95–98.
[11] G. Christen, R. Steffen, G. Renger, Delayed fluorescence emitted from light harvesting complex II and photosystem II of higher plants in the 100 ns–5 μs time domain, FEBS Lett. 475 (2000) 103–106.
[12] M. Grabolle, H. Dau, Energetics of primary and secondary electron transfer in Photosystem II membrane particles of spinach revisited on basis of recombination-fluorescence measurements, Biochim. Biophys. Acta 1708 (2005) 209–218.
[13] V. Goltsev, P. Chernev, I. Zaharieva, P. Lambrev, R.J. Strasser, Kinetics of delayed chlorophyll *a* fluorescence registered in milliseconds time range, Photosynth. Res. 84 (2005) 209–215.
[14] M. Seibert, R. Picorel, A.B. Rubin, J.S. Connolly, Spectral, photophysical, and stability properties of isolated photosystem II reaction center, Plant Physiol. 87 (1988) 303–306.
[15] B. Crystall, P.J. Booth, D.R. Klug, J. Barber, G. Porter, Resolution of a long lived fluorescence component from D1/D2/cytochrome *b*-559 reaction centres, FEBS Lett. 249 (1989) 75–78.
[16] P.J. Booth, B. Crystall, L.B. Giorgi, J. Barber, D.R. Klug, G. Porter, Thermodynamic properties of Dl/D2/cytochrome *b*-559 reaction centres investigated by time-resolved fluorescence measurements, Biochim. Biophys. Acta 1016 (1990) 141–152.
[17] P.J. Booth, B. Crystall, I. Ahmad, J. Barber, G. Porter, D.R. Klug, Observation of multiple radical pair states in photosystem 2 reaction centers, Biochemistry 30 (1991) 7573–7586.
[18] L. Konermann, G. Gatzen, A.R. Holzwarth, Primary processes and structure of the photosystem II reaction center: 5. Modeling of the fluorescence kinetics of the D1–D2–cyt–*b*559 complex at 77 K, J. Phys. Chem. B 101 (1997) 2933–2944.
[19] N. Murata, K. Satoh, Absorption and fluorescence emission by intact cells, chloroplasts, and chlorophyll–protein complexes, in: Govindjee, J. Amesz, D.C. Fork (Eds.), Light Emission by Plants and Bacteria, Academic Press, London, 1986, pp. 137–159.
[20] R. van Grondelle, J.P. Dekker, T. Gillbro, V. Sundstrom, Energy transfer and trapping in photosynthesis, Biochim. Biophys. Acta 1187 (1994) 1–65.
[21] Govindjee, Chlorophyll *a* fluorescence: a bit of basics and history, in: G.C. Papageorgiou, Govindjee (Eds.), Chlorophyll *a* Fluorescence: A Signature of Photosynthesis, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2004, pp. 1–42.

[22] E.G. Andrizhiyevskaya, A. Chojnicka, J.A. Bautista, B.A. Diner, R. van Grondelle, J.P. Dekker, Origin of the F685 and F695 fluorescence in Photosystem II, Photosynth. Res. 84 (2005) 173–180.
[23] H.Y. Nakatani, B. Ke, E. Dolan, C.J. Arntzen, Identity of the photosystem II reaction center polypeptide, Biochim. Biophys. Acta 765 (1984) 347–352.
[24] C.P. Rijgersberg, J. Amesz, Changes in light absorbance and chlorophyll fluorescence in spinach chloroplasts between 5 and 80 K, Biochim. Biophys. Acta 502 (1978) 152–160.
[25] D.A. Berthold, G.T. Babcock, C.F. Yocum, A highly resolved, oxygen-evolving photosystem II preparation from spinach thylakoid membranes: EPR and electron-transport properties, FEBS Lett. 134 (1981) 231–234.
[26] T.M. Bricker, J. Morvant, N. Masri, H.M. Sutton, L.K. Frankel, Isolation of a highly active photosystem II preparation from *Synechocystis* 6803 using a histidine-tagged mutant of CP47, Biochim. Biophys. Acta 1409 (1998) 50–57.
[27] O. Nanba, K. Satoh, Isolation of a photosystem II reaction center consisting of D-1 and D-2 polypeptides and cytochrome *b*-559, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 109–112.
[28] T. Tomo, E. Hirano, J. Nagata, K. Nakazato, Pigment exchange of photosystem II reaction center by chlorophyll *d*, Photosynth. Res. 84 (2005) 77–83.
[29] I. Ikegami, S. Katoh, Enrichment of Photosystem I reaction center chlorophyll from spinach chloroplasts, Biochim. Biophys. Acta 376 (1975) 588–592.
[30] X.-S. Tang, B.A. Diner, Biochemical and spectroscopic characterization of a new oxygen-evolving photosystem II core complex from the cyanobacterium *Synechocystis* PCC 6803, Biochemistry 33 (1994) 4594–4603.
[31] M. Rögner, P.J. Nixon, B.A. Diner, Purification and characterization of photosystem I and photosystem II core complexes from wild-type and phycocyanin-deficient strains of the cyanobacterium *Synechocystis* PCC 6803, J. Biol. Chem. 265 (1990) 6189–6196.
[32] B. Loll, J. Kern, W. Saenger, A. Zouni, J. Biesiadka, Towards complete cofactor arrangement in the 3.0 Å resolution structure of photosystem II, Nature 438 (2005) 1040–1044.
[33] S. Itoh, M. Iwaki, Delayed fluorescence in Photosystem I enhanced by phylloquinone (vitamin K-1) extraction with ether, Biochim. Biophys. Acta 934 (1988) 32–38.
[34] M. Iwaki, M. Mimuro, S. Itoh, Fluorescence of P700 and antenna chlorophylls in photosystem I particles containing 11 chlorophylls/P700, Biochim. Biophys. Acta 1100 (1992) 278–284.
[35] V.I. Prokhorenko, A.R. Holzwarth, Primary processes and structure of the photosystem II reaction center: a photon echo study, J. Phys. Chem. B 104 (2000) 11563–11578.
[36] R.N. Frese, M. Germano, F.L. de Weerd, I.H.M. van Stokkum, A.Y. Shkuropatov, V.A. Shuvalov, H.J. van Gorkom, R. van Grondelle, J.P. Dekker, Electric field effects on the chlorophylls, pheophytins, and β-carotenes in the reaction center of photosystem II, Biochemistry 42 (2003) 9205–9213.
[37] T. Noguchi, T. Tomo, C. Kato, Triplet formation on a monomeric chlorophyll in the photosystem II reaction center as studied by time-resolved infrared spectroscopy, Biochemistry 40 (2001) 2176–2185.
[38] A.W. Rutherford, Govindjee, Y. Inoue, Charge accumulation and photochemistry in leaves studied by thermoluminescence and delayed light emission, Proc. Natl. Acad. Sci. U. S. A. 81 (1984) 1107–1111.
[39] Y. Inoue, Photosynthetic thermo luminescence as a simple probe of photosystem II electron transport, in: J. Amesz, A.J. Hoff (Eds.), Biophysical Techniques in Photosynthesis, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1996, pp. 93–107.
[40] I. Vass, Govindjee, Thermoluminescence from the photosynthetic apparatus, Photosynth. Res. 48 (1996) 117–126.
[41] B. Ke, Photosynthesis, Photobiochemistry and Photobiophysics, Advances in Photosynthesis, vol. 10, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2001.