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Picosecond time-resolved fluorescence spectra of photosystem I and II in *Chlorella pyrenoidosa*

I. Yamazaki, M. Mimuro*, N. Tamai, T. Yamazaki and Y. Fujita*

Institute for Molecular Science, Myodaiji, Okazaki 444, and **National Institute for Basic Biology, Myodaiji, Okazaki 444, Japan*

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Picosecond time-resolved fluorescence spectra emitted from intact cells of the green alga *Chlorella pyrenoidosa* have been measured by means of a new detection technique using a microchannel-plate photomultiplier. A fluorescence band (F700) was observed at 690–730 nm in the initial time region (0–180 ps), in addition to the well-known spectrum (F685) of photosystem II (PS II)-chlorophyll *a* (Chl *a*) with a peak at 685 nm. F700 decays rapidly with lifetime of 104 ps, while F685 decays much more slowly in bi-exponential form with lifetimes of 0.64 and 1.7 ns. Appearance of F700 is independent of closure of the reaction center II (RC II). F700 is thus assigned to the fluorescence from PS I-Chl *a*, whose decay is governed by a fast energy transfer process from the antenna Chl *a* of PS I to P700 of RC I.

Chlorella pyrenoidosa *Photosystem I* *Time-resolved fluorescence spectrum* *Chlorophyll-protein complex* *Energy transfer* *Photosynthesis*

1. INTRODUCTION

Primary processes of the photosynthesis in plants are characterized by highly efficient absorption and subsequent transfer of photo-excitation energy from the light-harvesting pigment system to the reaction centers. These ultrafast processes of energy transport have been studied on the basis of picosecond time-resolved fluorescence spectroscopy [1–5]. In green algae, both PS I and PS II consist of antenna Chl *a* proteins and reaction center [6]. It has long been considered that the photosynthetic organisms in plants emit fluorescence almost exclusively from PS II–Chl *a*, and the fluorescence from PS I–Chl *a* is extremely weak so that it is negligible for most purposes. Very recently, authors in [7–10] dealt with the picosecond fluorescence decay curves of green algae and chloroplasts at room temperature, and demonstrated that the fast decay components with the 90 ps lifetime is attributed to the fluorescence emission from Chl *a*-protein complexes coupled with PS I.

We have examined picosecond time-resolved fluorescence spectra of the phycobilin–Chl *a* pigment system at room temperature, and have provided evidence for the successive energy transfer from the outer surface to the inner core of phycobilisome and to PS II–Chl *a* in membranes [5]. This letter reports the fluorescence spectrum from PS I–Chl *a* and its decay kinetics in intact cells of *Chlorella pyrenoidosa* at room temperature.

Abbreviations: PS, photosystem; RC, reaction center; Chl *a*, chlorophyll *a*; DCMU, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea

2. EXPERIMENTAL

The green alga *C. pyrenoidosa* (C-105, IAM Collection, University of Tokyo) was grown autotrophically under continuous illumination with incandescent light (2.5 W/m^2) [5]. Air containing 0.5% CO_2 was continuously supplied. Cells at the late exponential growth phase were used for measurements. Picosecond time-resolved fluorescence spectra were measured with a synchronously

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pumped, cavity-dumped dye laser and time-correlated photon-counting system [5]. Here, a microchannel-plate photomultiplier (Hamamatsu R1564U) was used to obtain an instrumental response function with 50 ps pulse width (fwhm) for the scattered laser light.

3. RESULTS AND DISCUSSION

Fig.1 shows time-resolved fluorescence spectra of *C. pyrenoidosa* for the time region 0–180 ps, together with the spectrum at 1.1 ns. A fluorescence band with a peak at 685 nm, F685, appears throughout the time range concerned and corresponds to the well-known fluorescence spectrum of PS II–Chl *a* [11,12]. Apart from the main fluorescence band, it is seen by reference to the 1.1 ns spectrum that the spectra in the initial time region (0–180 ps) are enhanced in intensity around 700 nm, indicating the existence of an additional band. After 200 ps, the spectrum no longer changes with time. The difference spectra between the spectra in the 0–150 ps time region and that at 1.1 ns show a new fluorescence band centered at 700 nm, as shown in fig.1. Fluorescence decay curves depend largely on the monitoring wavelength. Typical decay curves are shown in fig.2. The decay curves monitored at 705 and 735 nm are recognized as consisting of 3 exponential decays with lifetimes of 104 ± 26, 642 ± 83 and 1725 ± 59 ps, whereas at 685 nm it consists of middle and slow components. The fast decay component appears only in the decays monitored at 690–730 nm, and corresponds to the new fluorescence bands, F700. F700 appears independent of closure of RC II by adding DCMU or by increasing the intensity of the excitation laser pulse, while F685 is strongly affected by the PS II state (vide infra). From these features, we assign F700 to the fluorescence originating from PS I–Chl *a*.

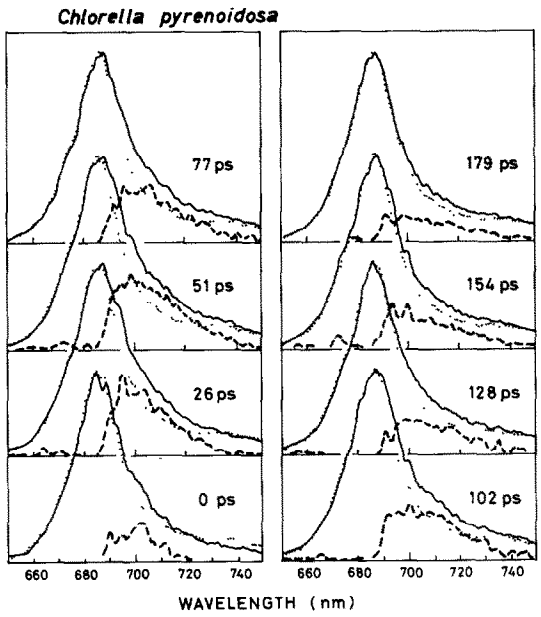

Fig.1. Time-resolved fluorescence spectra of *Chlorella pyrenoidosa* in the 0–180 ps time region (——) and at 1.1 ns (···), obtained by excitation at 630 nm. The highest intensities are normalized to a common value. Difference spectra (– – –) between the 1.1 ns spectrum and the respective spectra are shown after 2-fold expansion. Time zero corresponds to the time in which the excitation laser pulse reaches maximum intensity.

Green algae as well as higher plants exhibit at 77 K a fluorescence spectrum consisting of 3 bands, i.e., F685 and F695 due to PS II and F720 or F730 due to PS I [11–16].

Correlation between the spectra at 77 k and at room temperature is not straightforward; the spectral component corresponding to F720 or F730 at 77 K does not appear in any time region here. Recent studies in [17,18] are worthy of note. These authors examined the fluorescence emission from the RC I complex, and observed 3 fluorescence bands, F675, F655 and F705. Of these 3 fluorescence emissions, F705 was found to undergo specific changes depending on the redox condition of the primary electron acceptor of PS I, A_1; the intensity of F705 is increased as A_1 is reduced. They assigned F705 to an emission from excited Chl *a* associated closely with P700, resulting from charge recombination between $P700^+$ and A^- in PS I. The two fluorescence bands, F705 and F700, are reasonably regarded as identical in their origins and band locations, indicating that they are the same in nature. Contrary to the case of authors in [17,18], however, F700 here may not be a delayed fluorescence due to charge recombination, since RC I is open under our experimental conditions.

The present results and the above assignment for F700 are in accord with recent studies by others [7–10] who dealt with the fluorescence decay

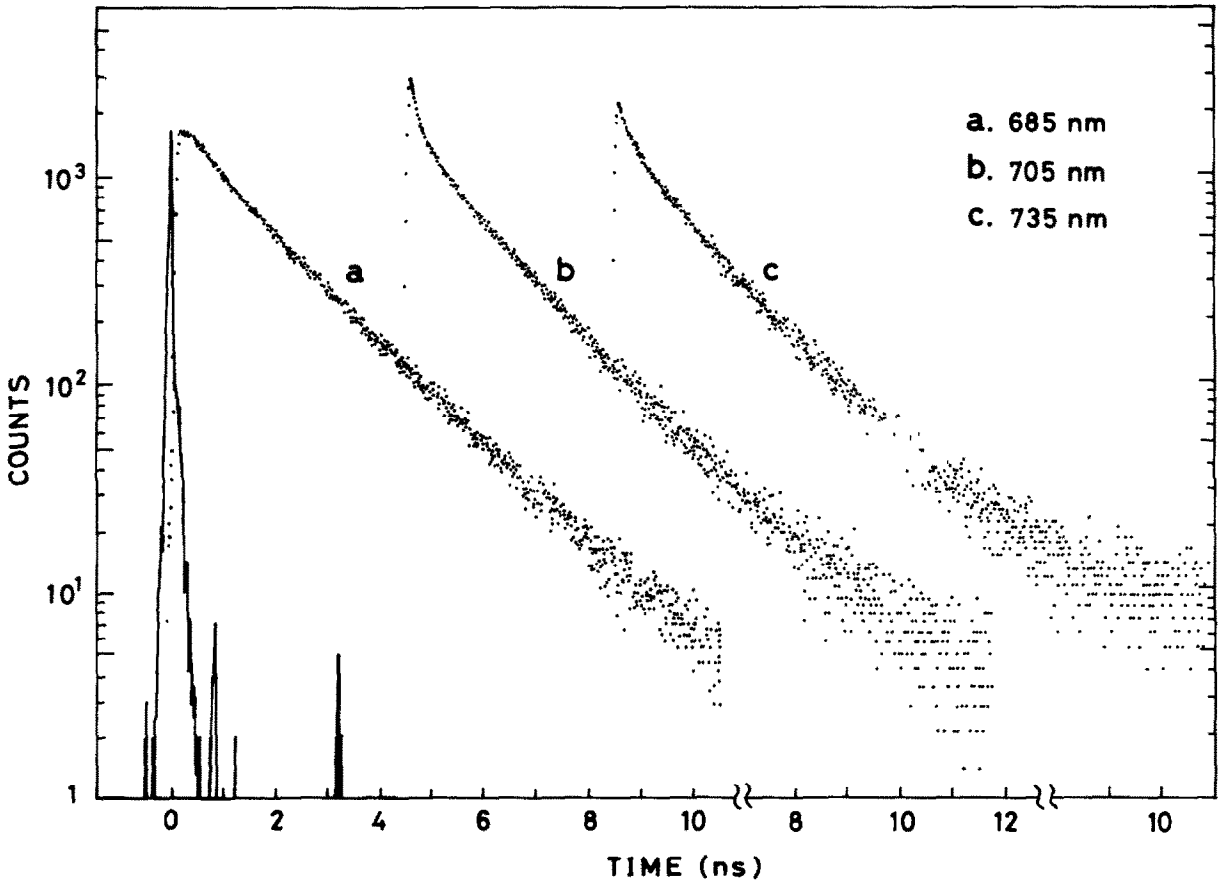

Fig.2. Fluorescence decay curves (···) and an instrumental response function of the excitation laser pulse (——). Excitation laser wavelength is 630 nm, and monitoring wavelengths are shown in the figure. These decay curves monitored at different wavelengths can be analyzed in terms of 3 exponential decays with lifetimes of τ_{fast} = 104 ± 26 ps, τ_{mid} = 642 ± 83 ps and τ_{slow} = 1725 ± 59 ps, with different intensities depending on the monitoring wavelength.

curves for some algae by using the picosecond laser and photon-counting detection system. Authors in [7,8] analyzed the decay curves of *Chlorella vulgaris* as a sum of 3 exponential decays with lifetimes of 0.1, 1.2–1.3 and 2.1–2.4 ns. The fast component was furthermore resolved into two components with lifetimes of 80 and 180 ps. Particularly, the wavelength distribution of amplitudes of the 80 ps component, which is different from those of the middle and slow components, shows the maximum around 700 nm. The spectrum shown in fig.1 can be regarded as corresponding to the 80 ps component. In our time-resolved spectrum, however, the 180 ps component could not be resolved. Some complexity remains regarding the spectral band position. According to the results in [9] for the red alga *Porphyra perforata,* the corresponding fluorescence band due to Chl *a* antennae coupled to PS I is located at a much longer wavelength with a peak at 730 nm. Further work is necessary for confirming the fluorescence band structure of PS I–Chl *a*.

F700 decays in single exponential form with a 100 ps lifetime, and F685 decays much more slowly in bi-exponential form. As pointed out previously, the fluorescence decay behavior of F685 varies with the excitation conditions. On increasing the intensity or repetition rate of the excitation laser pulses, the intensity of the slow component increases significantly, leaving their lifetimes practically constant. These features are consistent with the reaction mechanism for closed RC II proposed

in [19] and [20]. In the closed RC II where the electron acceptor Q is reduced, charge recombination takes place between $P680^+$ and the pheophytin anion (Ph^-), and regenerates fluorescent species of antenna Chl *a* from which the delayed fluorescence occurs. The slow decay component originates from the charge recombination in closed RC II, the lifetime of which is governed by the radiative and nonradiative transition rate of antenna Chl *a*, $k_{F685} = 0.6\times10^9\ s^{-1}$. The middle decay component arises from the antenna Chl *a* of open RC II, its lifetime depending on the rate constant of the energy transfer from antenna Chl *a* to P680, $k_{E2} = 1.6\times10^9\ s^{-1}$. On the other hand, the fast decay component is associated with PS I-Chl *a;* the inverse of its lifetime corresponds to the rate constant of energy transfer from antenna Chl *a* to P700 in PS I, $k_{E1} = 1\times10^{10}\ s^{-1}$. From an analysis of laser-intensity dependence and laser-frequency dependence of the fluorescence decay, the rate constant of the reverse process from the closed RC II to the open RC II is estimated to be $k_R = 5\times10^4\ s^{-1}$ [21].

The light-harvesting apparatus and reaction centers in green algae and higher plants consist of various kinds of chlorophyll-protein complex, and the excitation energy is transferred successively among them. The present results suggest that the photosynthetic pigment system in vivo emits fluorescence predominantly from the terminal Chl *a*-protein coupled closely with the respective reaction centers of PS I and PS II, i.e., F700 and F685, respectively.

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