

# Excitation energy transfer in spinach chloroplasts

## Analysis by the time-resolved fluorescence spectrum at $-196^{\circ}\text{C}$ in the picosecond time range

Mamoru Mimuro\*, Naoto Tamai, Tomoko Yamazaki and Iwao Yamazaki

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

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Excitation energy transfer in spinach chloroplasts was investigated by the time-resolved fluorescence spectrum at  $-196^{\circ}\text{C}$  with a time resolution better than 10 ps. The characteristic fluorescence bands belonging to each photosystem (PS) were clearly observed. In PS I, two new maxima were found at 690 and 705 nm, both of which showed very short lifetimes, and at least two other fluorescence bands were present at wavelengths longer than 700 nm. PS II particles showed the maximum at 680 nm just after the excitation pulse. The maximum shifted to 685 nm within 160 ps, and further to 695 nm within 350 ps. The energy flow in each PS was discussed based on the time behaviour of the components.

Chlorophyll *a*; Energy transfer; Fluorescence; Photosynthesis; Picosecond spectroscopy

### 1. INTRODUCTION

In the analysis of energy transfer in photosynthetic pigments, fluorescence has been used as an essential index. Time-resolved fluorescence spectra will give direct evidence for the energy flow among photosynthetic pigments. We have shown time-resolved fluorescence spectra of cyanobacteria, red alga [1,2] and the green alga *Chlorella pyrenoidosa* at room temperature [3]. Lowering of the temperature to that of liquid nitrogen is known to bring about the enhancement of the fluorescence intensity of PS I chl *a*, as well as better resolution of wavelength. Thus, we measured the time-resolved fluorescence spectrum at  $-196^{\circ}\text{C}$  of spinach chloroplasts and their subparticles under

the excitation condition of a low photon flux. Results showed the presence of a few new fluorescence components in PS I and the time behaviour of various components of both PSs.

### 2. MATERIALS AND METHODS

Spinach chloroplasts were isolated basically following the method of Jensen and Bassham [4]. The isolation medium contained 50 mM Tricine-NaOH (pH 7.5), 0.33 M sucrose, 10 mM NaCl and 2 mM  $\text{MgCl}_2$ . Subparticles were separated by a combination of mechanical disruption and differential centrifugation (cf. [5]).

The purity of the PS I and II particles was estimated based on the steady-state fluorescence spectrum at  $-196^{\circ}\text{C}$ . We made three assumptions: (i) PS I shows only the 735 nm fluorescence and PS II shows the 685 and 695 nm fluorescence components; (ii) by the excitation at 435 nm, the fluorescence intensity from chloroplasts can be expressed as the linear combination of the intensity from both PSs based on the unit amount of

Correspondence address: M. Mimuro, National Institute for Basic Biology, Myodaiji, Okazaki, Aichi 444, Japan

**Abbreviations:** FWHM, full width half maximum; PEG, polyethylene glycol; PS, photosystem; RC, reaction center

chlorophyll; (iii) the stoichiometry of PS I and II is unity in chloroplasts. The ratios of fluorescence intensity at 735 nm to that at 695 nm were 2.90, 13.9 and 1.05 for chloroplasts, PS I and II particles, respectively. These values indicate that the purity of our PS I and II particles was about 84 and 73%, respectively.

Time-resolved fluorescence spectra were measured with the apparatus reported in [1,2]. The excitation pulse was obtained from an Ar<sup>+</sup>-pumped dye laser (630 nm, pulse width 6 ps (FWHM) and intensity,  $10^8$ – $10^9$  photons/cm<sup>2</sup>). Fluorescence was detected by a time-correlated single photon counting system. The spectral sen-

sitivity of the apparatus was not corrected. The samples were dissolved in the buffer (50 mM Tricine-NaOH (pH 7.5), 0.33 M sucrose, 10 mM NaCl and 2 mM MgCl<sub>2</sub>) which contains PEG 4000 (final concentration, 15%) to obtain homogeneous ice at liquid nitrogen temperature.

### 3. RESULTS

In chloroplasts, the maximum was observed at 680 nm just after the excitation (fig.1A). The maximum shifted to 685 nm within 160 ps. At this time, the 695 nm fluorescence band was not significant. Further red shift to 695 nm was com-

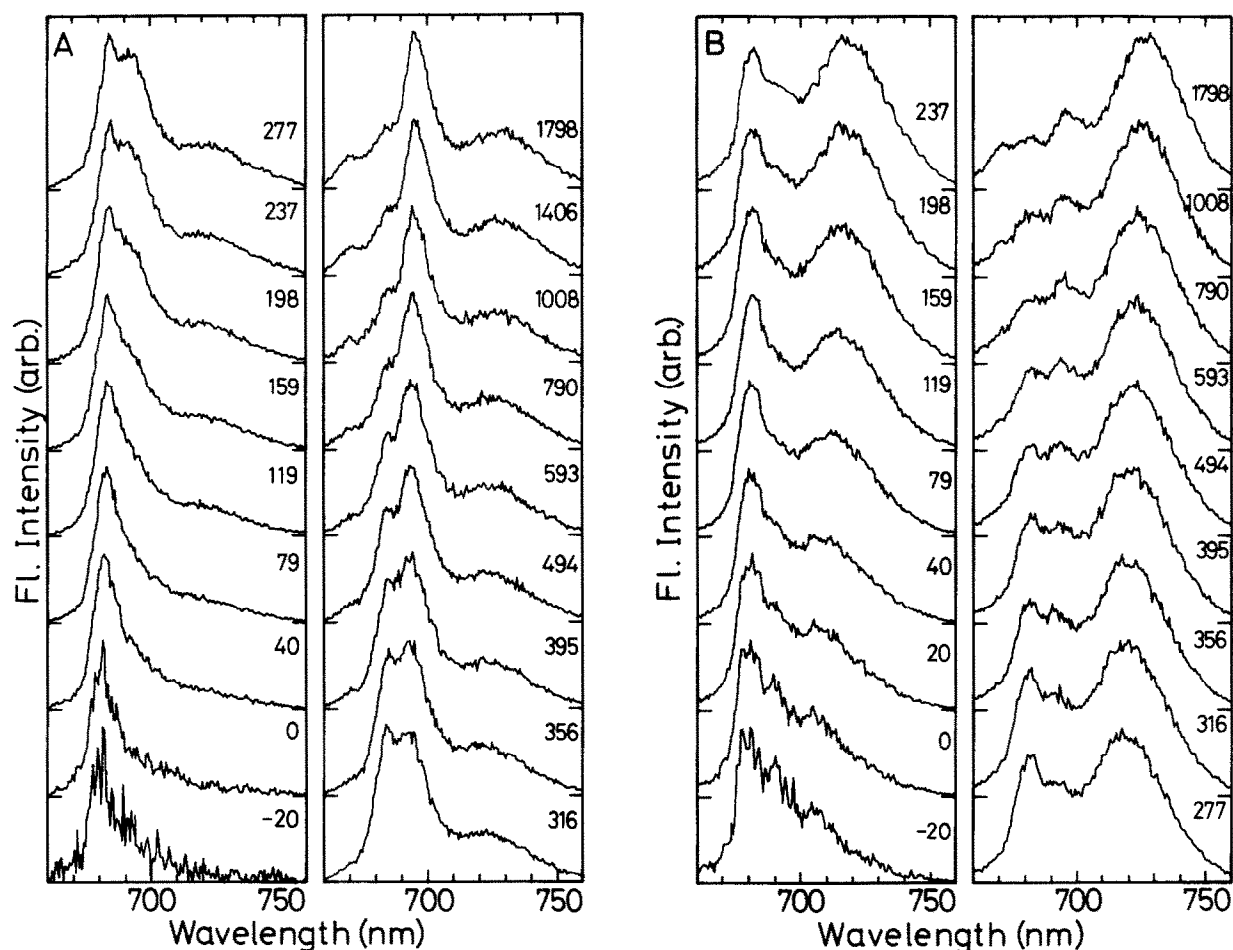


Fig.1. Time-resolved fluorescence spectra of spinach chloroplasts (A) and of PS I particles (B) at  $-196^{\circ}\text{C}$ . Samples were excited at 630 nm, with a duration of 6 ps. Fluorescence was measured through a monochromator (half bandwidth, 2 nm). Spectra were normalized to the maximum intensity in each spectrum. Numbers indicate the time after laser pulse in ps. For details, see section 2.

pleted within 350 ps and this component was dominant for longer than 2 ns. In the wavelength region longer than 700 nm, a distinct maximum was not found at the time close to the excitation pulse. However about 100 ps after the pulse, a small band appeared around 710 nm, and it became significant with time, accompanying the red shift, finally to give the maximum around 728 nm. Since the spectral sensitivity of the detector system drops sharply in the wavelength region longer than 700 nm, the 728 nm maximum is expected to be located at a longer wavelength, probably corresponding to the 736 nm fluorescence detected by steady-state measurements.

The decay kinetics around 680 nm was skewed, indicating the presence of a short lifetime component. At 695 nm, almost single exponential decay was observed, which gives a lifetime of 1.46 ns. At the wavelengths longer than 700 nm, the overall decay became fast, suggesting that the short lifetime component(s) is present in this wavelength region (not shown).

Judging from the locations of the maxima, the 680 nm fluorescence component was LHC II [6], and the 685 and 695 nm components were assigned as PS II chl *a* [7]. The fluorescence longer than 700 nm most probably originates from PS I chl *a*. Difference in the composition of fluorescence components was clear in the spectra of each subparticle.

In the case of PS II particles, essentially the same time behaviour of the 680, 685 and 695 nm components was observed as in the case of chloroplasts (not shown). The fluorescence intensity in the wavelength region longer than 700 nm was low, indicating that these arise from PS I chl *a*. The decay kinetics at particular wavelengths were almost the same as in chloroplasts.

PS I particles showed the presence of a few new fluorescence bands which were hardly detected in chloroplasts or PS II particles. Just after the excitation pulse, three maxima were found at 680, 690 and 705 nm (fig.1B). The first band, whose relative intensity was lower than that in chloroplasts (fig.1A), originates from LHC II contaminating the PS I particles. The latter two bands probably originate from PS I chl *a*. The 690 nm band disappeared immediately so that the maximum was observed only in the initial time span. In this wavelength region, the 695 nm band was clear

at a later time (fig.1B, at 500 ps). Since the maximum was located at significantly longer than 690 nm, the 695 nm band can be assigned as the PS II chl *a*. The location of fluorescence maximum at longer than 700 nm was not constant. The red shift lasted for over 1.2 ns after the excitation, and finally reached 728 nm. This shift indicates the progressive increase in the intensity of the longer wavelength component(s). Therefore, the number of fluorescence components in this wavelength region was not determined only by these spectra.

The lifetime of the main component at 735 nm was about 2.17 ns, whereas that at 720 nm was 1.45 ns. Around 710 nm, a component with a much shorter lifetime was observed (not shown). These results clearly indicate the presence of at least three fluorescence components in the wavelength region longer than 700 nm in PS I.

#### 4. DISCUSSION

In the steady-state fluorescence spectrum of spinach chloroplasts at  $-196^{\circ}\text{C}$ , at least five components are known with the maxima at 680 [6], 685 [7], 695 [7], 720 [8] and 735 nm [7]. These origins are assigned as LHC II, PS II, PS II, PS I and PS I, respectively. Our analysis confirmed the presence of these five components and further indicated the two new fluorescence components at 690 and 705 nm. These are assigned as PS I chl *a*, because these are found only in PS I particles. These bands are also detected in PS I particles at room temperature [9], and their lifetimes were very short under both temperature conditions. This clearly indicates that these components are functioning, and are not artificial chlorophyll forms which are expected to have long lifetimes, if present.

In PS II, the energy transfer occurred from LHC II to the 685 nm component, and further to the 695 nm component, as indicated by steady-state measurements [7]. On the other hand, in PS I, the 690 and 705 nm components have very short lifetimes, which were comparable to that of LHC II. This suggests that these two components are the intrinsic antenna of PS I and that neither is the energy acceptor of LHC II or PS II chl *a* (the 685 nm fluorescence component). The energy is most probably transferred from the 690 nm component to the reaction center I (RC I). The fast

decay of the 705 nm component suggests the energy flow from this pigment to RC I, however, the reversed energy levels do not necessarily support this flow. The 720 and 735 nm components are most probably the energy sink of PS I at  $-196^{\circ}\text{C}$ , because of their longer lifetimes and the energy levels.

This study is the first demonstration of the time-resolved fluorescence spectrum at  $-196^{\circ}\text{C}$  of spinach chloroplasts under the conditions of a low photon flux, contrary to the previous measurements of decay kinetics under high flux conditions [10,11]. The energy flow in each PS became clear, however the energy flow from PS II to PS I was not shown. This is due to a lower intensity of PS I emission in the initial time range. This problem must be investigated further.

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