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SINGLE-PULSE PICOSECOND DETERMINATION OF 735 nm FLUORESCENCE RISETIME IN SPINACH CHLOROPLASTS

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1. Introduction

Within the last few years there have been extensive investigations of the fluorescence from photosynthetic systems using picosecond lasers as excitation sources [1–11]. Such studies have established that the quenching of the prompt fluorescence, with increasing incident excitation intensity employing a single pulse, arises primarily from singlet–singlet exciton annihilation [2,3,7–9]. When the mode of excitation consists of a train of pulses or of a microsecond laser pulse, there exists additional quenching of the fluorescence quantum yield arising from long-lived quenchers, presumably triplet excitons [2,3,10], which either arise from intersystem crossing from the singlet manifold or from random recombination of electrons and holes formed from the autoionization final state channel of singlet fusion [8]. Recently, Geacintov et al. [8] have shown that the quantum yield quenching at emission wavelengths of 685 nm and 735 nm were identical, within experimental error, when single picosecond excitation pulses were employed. This identity in the quenching curves was interpreted in terms of the tripartite fluorescence model with bimolecular singlet fusion occurring exclusively within the light harvesting antenna system. Strong support for this interpretation was provided by the observation of an intensity-independent (for intensities below 10^{15} photons cm^{-2} per pulse) lifetime for the 735 nm emission [11]. Presumably, singlet fusion reactions are either inoperative or inefficient within the PS I antenna chlorophyll molecules, at least for intensities below 10^{15} photons cm^{-2} per pulse. Within the light harvesting and PS II antenna pigments which give-rise to the 685 nm emission, singlet exciton fusions do give rise to a strong decrease of the fluorescence lifetime with increasing intensity. It was concluded that the pigments which are responsible for the 735 nm emission derive their energy by singlet exciton transfer from the light harvesting system and not by direct photon absorption at 530 nm. In this paper we measure the risetime for the 735 nm emission and identify this time lag with the transfer rate from the light harvesting system to the PS I pigment molecules which give rise to the 735 nm fluorescence band at low temperatures.

2. Materials and methods

The experimental arrangement for fluorescence lifetime measurements was similar to that reported previously [3]. A 1060 nm, 30 ps pulse was selected from the pulse train emitted by a modelocked Nd:YAG laser, frequency shifted to 530 nm by passage through a KDP crystal, and then was allowed to excite the spinach samples. The samples, chloroplasts prepared from spinach leaves as described in [12], were

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contained in a high optical quality 2 mm thick cuvette, maintained at 77°K within an optical dewar. The sample was illuminated uniformly, and the fluorescence was collected onto the slit of an Electrophotonics ICC 512 (S-20 response) streak camera with *f*/1 optics. The temporal rise and decay profiles for both the 690 nm and 735 nm fluorescence were obtained at 77°K. This was accomplished by using a narrow band pass filter at 735 nm to isolate the PS I fluorescence, whereas the PS II and light harvesting antenna pigment emission was differentiated by means of a 690 nm narrow band pass filter. In addition, appropriate filters further rejected the 530 nm radiation from the streak camera. Fluorescence streaks were imaged onto a SSR silicon vicidon optical multichannel analyzer and then displayed on an oscilloscope after each shot. Shots could be accumulated on a Nicolet signal averager, thereby allowing detection at lower excitation intensities and hence averting exciton annihilation effects and also greatly improving the signal-to-noise ratio. Because there is considerable variation from shot to shot in the start of the streak due to electrical jitter, a reference point in time was established by allowing a weak green pulse, which bypasses the sample, to enter the streak camera a few hundred picoseconds before the arrival of the fluorescence. The zero time for the fluorescence onset and the excitation pulseshape characteristics about the zero time position could be determined by examining 530 nm light scattered off a dummy sample cuvette.

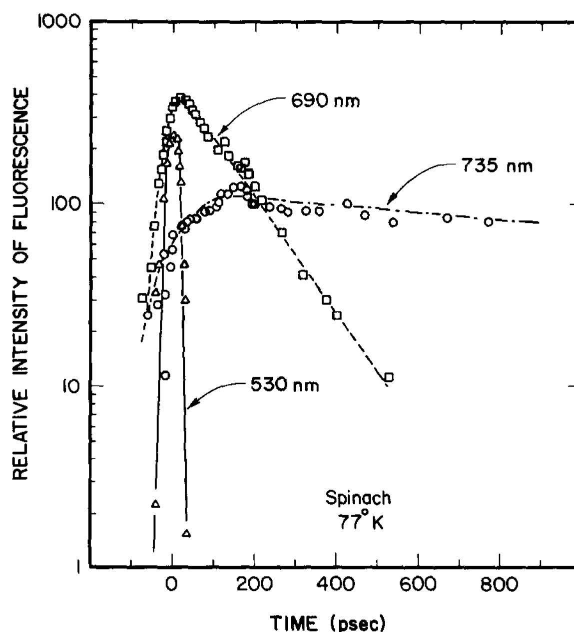


Fig.1. Time resolved fluorescence from spinach chloroplasts at a temperature of 77°K and an incident intensity of 2×10^{14} photons cm^{-2}. Emission at 690 nm rises promptly, following 530 nm excitation pulse, whereas emission at 735 nm rises much more slowly.

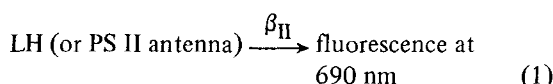
3. Results

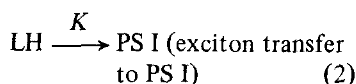
As shown in fig.1, the 735 nm emission continues to rise after termination of the excitation pulse, whereas the 690 nm emission rises abruptly with characteristics expected from the pulse shape. The risetime of the 735 nm fluorescence, taken from the 10% to 90% of peak intensity, was measured to be 140 ± 40 ps, an average obtained from six separate experimental runs, each of about 30 shots. This risetime was observed over a wide range of incident intensities, from 10^{13} to 2×10^{14} photons cm^{-2}. In fig.1 the risetime of the 735 nm emission is comparable to the falltime of the 690 nm emission. However, at intensities of 10^{13} photons cm^{-2} or lower, where the decay time of the 690 nm emission was measured to be about 900 ps, the risetime of the 735 nm emission remained near 140 ps. Thus, the 735 nm risetime does not correspond to the decay of the 690 fluorescence. The decay time of the 735 nm emission at low intensities was near to 1.5 ns, close to that reported previously. The risetime result at 735 nm, however, is in sharp conflict with the results of Yu et al. [13] obtained with high intensity pulse trains. We interpret their measurement of an abrupt rise as due to the generation of long-lived transient quenchers [14] by the pulse train and subsequent annihilation effects. Nor does our data at 690 nm agree with that observed previously by Seibert and Alfano [15,16]. Their identification of two peaks in their data [15], not observed by us, as originating from PS I (risetime $\leq$10 ps, lifetime $\leq$10 ps) and PS II (risetime 90 ps, lifetime 210 ps) leads to opposite conclusions than does this paper. Their data, like that of Yu et al. [13] was also obtained with high intensity pulse train

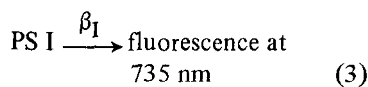
excitation and has lately come under much criticism [2,3,8,13,14].

4. Discussion

The slower risetime of the 735 nm PS I fluorescence as compared to the risetime of the 690 nm PS II and light harvesting (LH) pigment fluorescence is consistent with our previous conclusion [11] that the 735 nm emitting pigment system derives its energy by exciton transfer from the LH pigments. An expression for the time dependence of the 735 nm fluorescence can be derived based on these considerations and which can be summarized by the following steps:







If we denote the exciton density in PS I by n_I, and denote the exciton density within the LH pigments by n_{II}, then the time dependence of these quantities is described by

$$\frac{dn_I}{dt} = K\, n_{II}(t) - \beta_I n_I(t) \qquad (4)$$

$$\frac{dn_{II}}{dt} = G(t) - (\beta_{II} + K) n_{II}(t) \qquad (5)$$

where $G(t)$ denotes the laser pulse generation source and K, β_I and β_{II} are the rate constants defined by (1) -(3). For the sake of simplicity, these equations do not include details of Kitajima and Butlérs tripartite fluorescence model such as separate emission bands from LH and PS II antenna pigments, energy transfer between these two systems [17,18], and the possibility that the 735 nm fluorescence is due to a chlorophyll from C-705 which derives its energy by exciton transfer from PS I antenna pigments [19].

Treating the incident source as a delta function, a good approximation because the risetime of the 735 nm emission is at least a factor of five-times the excitation pulse width, eq. (4) and eq. (5) are easily solved:

$$n_{II}(t) = n_{II}(0)\, e^{-(\beta_{II} + K)t} \qquad (6)$$

$$n_I(t) = \frac{K\, n_{II}(0)}{\beta_I - (K + \beta_{II})} \left[e^{-(\beta_{II} + K)t} - e^{-\beta_I t}\right] \qquad (7)$$

The first term of eq. (7) characterizes the risetime of the 735 nm fluorescence (140 ± 40 ps), while the second term characterizes the decay ($\beta_I^{-1} \approx 1.5$ ns).

Equations (6) and (7) are the most simple representations of the time dependence of the 685–690 nm and the 735 nm fluorescence emissions, respectively. The data in fig.1 indicates that $(\beta_{II} + K)^{-1}$ = 140 ± 40 ps, which implies, according to eq. (6), that the lifetime of the fluorescence at 690 nm should display the same time dependence. However, this is not the case, since the fluorescence decay time at ~690 nm is about 800 ps [20]. Furthermore, below intensities of ~10^{15} photons cm^{-2} per pulse, the 735 nm fluorescence risetime remains constant, within experimental error, while the lifetime of the 685–690 nm fluorescence varies strongly with intensity due to exciton annihilation.

At higher excitation intensities, when the quadratic term must be included in eq. (1) it can be shown [21] that the appropriate modification of eq. (6) and eq. (7) corresponds to replacing β_{II} by $\beta_{II} + c\gamma_{ss}\alpha I$ where γ_{ss} is the singlet fusion rate, α (cm^{-1}) is the absorption coefficient, I is the incident intensity (photons cm^{-2}), and c is a numerical constant $\simeq 0.6$. Hence when the excitation intensity of the single pulse obeys the equation

$$I > \frac{K - \beta_{II}}{c\gamma_{ss}\alpha}, \qquad (8)$$

a decrease in the risetime of the 735 nm fluorescence is predicted. We indeed observe such a decrease when the excitation intensity exceeds $I = 10^{15}$ photons cm^{-2} per pulse.

The lack of a correlation between the 735 nm fluorescence risetime and the 690 nm decay time for $I < 10^{15}$, and the decrease in the 735 nm risetime for $I > 10^{15}$ photons cm^{-2} per pulse, can be understood

in terms of the following model for the LH pigment system.

There are two types of LH pigments; in one of these denoted by LH(1), the chlorophyll molecules are tightly coupled to the PS I pigment system with $K > \beta_{II}$; i.e., the exciton lifetime is mainly determined by energy transfer to PS I, with $K^{-1} \approx 140$ ps. The fluorescence yield from this system is low.

The second type of LH pigments, LH(2), consists of chlorophyll molecules which are less tightly coupled to PS I (possible because of a large physical separation) and which decay mainly by the rate constant $\beta_{II} \simeq (800 \text{ ps})^{-1}$. The fluorescence yield from this system is relatively high. The 735 nm rise-time is thus unaffected by exciton annihilation within LH(1) up to intensities of $I \sim 10^{15}$ photons cm^{-2} per pulse when the annihilation rate begins to compete with the rapid LH(1) → PS I transfer rate characterized by the rate constant K.

This model of the fluorescence of spinach chloroplasts at low temperatures is depicted in fig.2. It is capable of accounting for all of the observations presented here, as well as for the identity of the quenching curves at 685 nm and 735 nm [11].

It is possible that LH(1) represents a relatively small fraction of the total light harvesting pigments. This is indicated by the fact that the 735 nm fluorescence reflects the exciton density within the light harvesting pigments [11], which is not primarily determined by the LH → PS I energy transfer rate constant K, but is determined by the unimolecular rate β_{II} and the bimolecular exciton annihilation rate. Thus, excitons appear to communicate between LH(1) and LH(2), but the LH(2) → LH(1) → PS I pathway does not characterize the major pathway for excitons within the overall LH pigment system.

The model shown in fig.2 predicts that careful measurements of fluorescence decay curves at low intensities of excitation (no bimolecular exciton annihilation) may reveal two components: a fast but weak component due to exciton decay by energy transfer from LH(1) → PS I characterized by a lifetime of K^{-1}, and a longer more intense component, which is characterized by β_{II}.

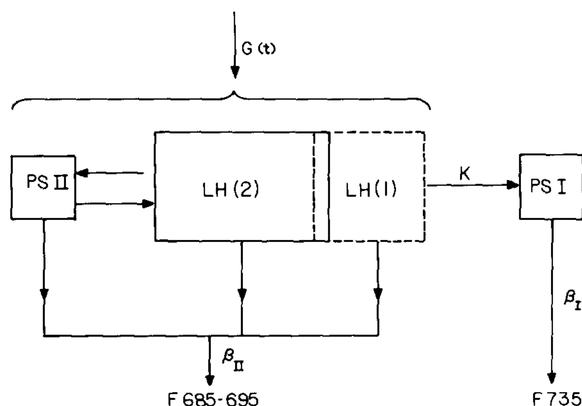


Fig.2. Modified Tripartite Fluorescence Model [17,18]. The source term G(t), excites both the light harvesting aggregate, LH, and the PS II chlorophyls. Fluorescence emission corresponding to LH, PS II, and PS I are at 685, 695 and 735 nm [16–18]; however, no differentiation is made between the PS II and LH emission in this work. The light harvesting pigment system is presumed to consist of two fractions: in LH(1) the light harvesting pigments are closely associated with PS I and energy transfer occurs with rate constant K; in LH(2) the pigments are not closely connected to PS I and excitons decay mainly with the rate constant β_{II}. The decay rate of the 735 nm fluorescence, which reflects the exciton density in PS I [17], is denoted by β_I.

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