

## Temperature dependence of the 735 nm fluorescence kinetics from spinach measured by picosecond laser-streak camera system

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### Summary

The 735 nm fluorescence lifetime ( $\tau$ ) and relative quantum yield ( $\phi$ ) of spinach were measured at 90 K as a function of excitation fluence. The 735 nm risetime, defined as the time required for the intensity to increase from 10 to 90% of its maximum value was measured to be  $19.6 \pm 3$  ps at 106 K for excitation fluence below  $10^{14}$  photons/cm<sup>2</sup>. The fluorescence quantum yield for wavelengths greater than 720 nm revealed no apparent intensity-dependent behaviour over the excitation fluence of  $10^{13}$  to  $10^{15}$  photons/cm<sup>2</sup>. The fluorescence decay profile, however, was found to change from a single exponential to a non-exponential behaviour above  $3 \times 10^{14}$  photons/cm<sup>2</sup> per pulse. These results are interpreted in terms of energy transfer from accessory pigments or optically excited antennae of PS I and a lack of energy transfer from the light harvesting 685 nm antennae to the C-705. The temperature dependence of the 735 nm fluorescence lifetime was measured and the excitation energy transfer from C-705 to P-700 reaction centers of PS I is discussed in terms of the electron multiphonon formalism of Jortner and Yomosa. From the analysis of the data we have obtained a mean phonon energy  $\langle \hbar\omega \rangle \sim 418$  cm<sup>-1</sup> and an electron-phonon coupling strength  $S = 3.7$ .

**735 nm fluorescence; temperature dependence; picosecond fluorescence spectroscopy; fluorescence risetime and decay times, energy transfer**

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*Abbreviations:* PS I, Photosystem I; PS II, Photosystem II; RC I, RC II reaction centers I and II.

## Introduction

The temporal evolution of the fluorescence from photosynthetic systems has been interpreted as indicative of the energy transfer occurring in the photosynthetic accessory pigment complex [1-3]. In particular, the fluorescence decay time has been associated with the time required for the absorbed optical energy to reach the photochemically active reaction center trap [2]. By following the energy transfer route to the photochemical trap, it is therefore possible to elucidate the mechanisms which determine its specific pathways and the participants involved in the transfer. Over the past twenty years, various spectroscopic studies have been undertaken over a wide range of temperatures in order to better understand the physical nature of the primary photosynthetic processes. Although the reactions taking place at low temperature are quite different from the physiological reactions which occur at room temperature, much valuable information has been obtained. In higher green plants the prominent fluorescence components are located at 685 nm at room temperature and at 685 nm, 695 nm, and 735 nm at low temperatures [2-5]. It is now generally accepted that the 685 nm, 695 nm and 735 nm components arise from chlorophyll *a* emission in the light harvesting antennae, PS II and PS I units, respectively [3-6].

Considerable progress in the understanding of the primary energy conversion mechanism in photosynthesis has been achieved through the use of picosecond fluorescence and absorption spectroscopy [7,8]. By studying the picosecond time-resolved temperature dependence of the fluorescence lifetimes at different emission wavelengths, one can obtain basic information on the energy transfer mechanisms and the pathways of energy migration to the traps. The 685 nm emission from the light harvesting pigments shows only a slight temperature dependence [6,9-11]. On the other hand, the intensity and lifetime of the 735 nm emission band is strongly dependent on temperature [6,9-11]. At room temperature, the yield of the 735 nm (PS I) fluorescence is so low that it is buried in the tail of the 685 nm emission band. As the temperature is lowered the 735 nm band appears and its lifetime increases dramatically. Paschenko et al. [12] have recently reported on the temperature dependence of the fluorescence lifetime from pea chloroplasts at various wavelengths in order to elucidate the role of these components in the energy transfer to RC I and RC II. Recently, Butler and coworkers [13-16] have shown that the 735 nm emission is associated with a small group of special chlorophyll pigments (*C*-705) which absorb at 705 nm and which are present at all temperatures. The fluorescence emission from *C*-705 is less intense at higher temperatures due to the lower activation energy required for energy transfer to the reaction center trap (*P*-700) [15,16]. On lowering the temperature, energy transfer from *C*-705 to *P*-700 becomes less probable due to the energy barrier of the *P*-700 level, resulting in an increase of the 735 nm emission [16].

The aim of this research note has been five-fold:

- (1) To measure the risetime of the 735 nm fluorescence emission at 106 K and at low excitation intensity (below  $10^{14}$  photons/cm<sup>2</sup>);
- (2) To measure the 735 nm fluorescence lifetime and quantum yield as a function of excitation fluence at 90 K;

- (3) To measure the temperature dependence of the 735 nm fluorescence lifetime;
- (4) To find the location and barrier height of the energy level of the trap *P*-700; and
- (5) To describe the data in terms of a multiphonon resonance excitation energy transfer from *C*-705 to *P*-700.

### Methods

The time-resolved fluorescence emission from an intact spinach leaf due to a single excitation flash was measured as a function of temperature with a picosecond streak camera and optical multichannel (SIT) video detection system. The experimental set-up, which has been previously described in detail [17,18] consists of a single 6 ps 530 nm excitation pulse produced from a Nd:glass laser by the process of second harmonic generation. The 530 nm pulse was used to excite the spinach leaf sample at a photon fluence ranging from  $10^{13}$  to  $10^{15}$  photons/cm<sup>2</sup>. The beam was collimated to a uniform spot size of 1 × 2 mm at the sample site. The sample was kept in the dark prior to and during the experiment in order to enable the experiment to be carried out in an open trap state. The time resolution of the system from 530 to 760 nm is  $\leq 12$  ps.

In the measurement of risetimes care must be taken in order to ensure the integrity of the ultimate time resolution of the streak image for the different spectral regions investigated. The risetime in this experiment was determined by measuring the time required for the intensity to increase from 10 to 90% of the maximum value. The relative fluorescence quantum yields were calculated as the total integrated area under the best fit curve, and normalized to the value of the quantum yield at low intensity,  $I \leq 3 \times 10^{13}$  photons/cm<sup>2</sup>. The spinach leaf was mounted on a flat aluminum plate and inserted in an optical dewar. The temperature of the sample was changed by flowing cooled nitrogen gas into the dewar, and the temperature at the sample site monitored with a copper-constantan thermocouple. Narrow band filters (FWHM  $\sim 10$  nm) and a Hoya colored filter (R72) isolated the 687 nm and 735 nm emission bands, respectively.

### Measurements

The low temperature fluorescence lifetime (90 K) from a green spinach leaf was measured with a 687 nm narrow band filter (FWHM  $\sim 10$  nm) at an average single pulse excitation intensity of  $4 \times 10^{14}$  photons/cm<sup>2</sup> per pulse. The decay was characterized by a single exponential behavior with a lifetime of  $227 \pm 25$  ps (10 measurements). The low temperature 687 nm decay time at this intensity is in agreement with the measurement of Campillo et al. [19] ( $1/e$  time 200 ps). This lifetime is also in close agreement with our measurement of the 685 nm room temperature fluorescence lifetime [20]. Measurements of the lifetime of the 685 nm component at room temperature and at low intensity have recently been reported by

Sauer and Brewington [21] who obtained 200 ps for spinach chloroplasts using the photon counting technique at an excitation intensity of  $10^7$  photons/cm<sup>2</sup>, and Pellegrino [20] who obtained  $\sim 200$  ps using streak camera detection and single pulse excitation at an intensity of  $\sim 10^{13}$  photons/cm<sup>2</sup> per pulse. The 685 nm lifetime measurement is also in agreement with the early results of Seibert and Alfano [22] and Yu et al. [10,23] at room temperature and 100 K. However, since the onset for exciton annihilation at room temperature occurs above  $3 \times 10^{14}$  photons/cm<sup>2</sup> per pulse, a slight quenching effect may be present, which can manifest itself in a change in the fluorescence decay profile producing in general a fast and slow component decay.

The risetime of the 735 nm fluorescence intensity measured as the time required for the fluorescence intensity to increase from 10 to 90% of its maximum value was found to be  $19.6 \pm 3$  ps at 106 K (7 measurements) at a photon fluence of  $< 10^{14}$  photons/cm<sup>2</sup>. The fluorescence lifetime for a green spinach leaf at 90 K for wavelengths greater than 720 nm measured as a function of excitation intensity is shown in Fig. 1. The fluorescence quantum yield at 90 K from the spinach leaf measured as a function of excitation intensity is shown in Fig. 2. Each data point displayed represents the average of 3 measurements and the error bars denote the extent of the standard deviation from the mean. The low temperature quantum yield shows no clear intensity dependent variation over the range of  $10^{13}$  to  $10^{15}$  photons/cm<sup>2</sup>.

At low intensity,  $I = 3.3 \pm 0.81 \times 10^{13}$  photons/cm<sup>2</sup> per pulse, the low temperature fluorescence lifetime decayed as a single exponential with a lifetime of  $1471 \pm 38$  ps (11 measurements). However, for  $I \geq 3 \times 10^{14}$  photons/cm<sup>2</sup> per pulse the fluores-

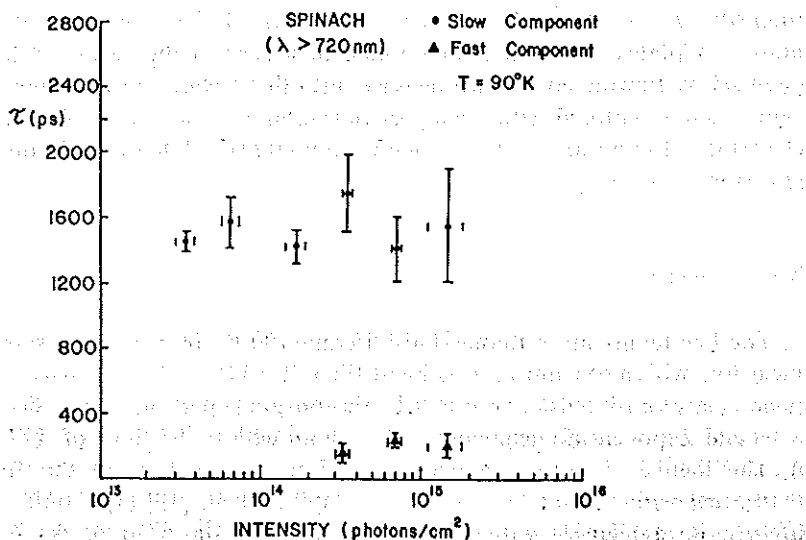


Fig. 1. Fluorescence lifetime from intact spinach leaf at 90 K for emission wavelengths  $> 720$  nm measured as a function of excitation pulse fluence.

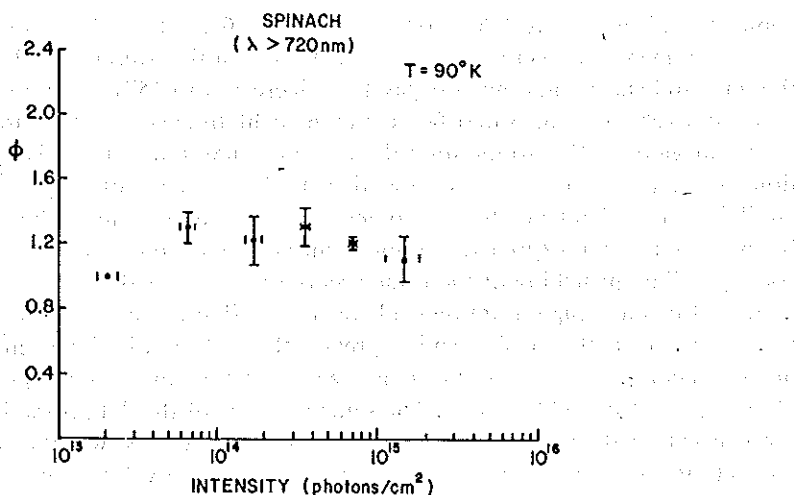


Fig. 2. Relative fluorescence quantum yield from spinach leaf at 90 K for emission wavelengths  $> 720$  nm measured as a function of excitation pulse fluence.

cence was found to decay non-exponentially. In particular it was found that the fluorescence intensity could be analyzed as the sum of two exponentials at  $I = 3.4 \pm 0.2 \times 10^{14}$  photons/cm<sup>2</sup> per pulse (3 measurements) a long component of  $1733 \pm 241$  ps and a short component of  $221 \pm 11$  ps (with relative amplitudes of  $0.74 \pm 0.06$  and  $0.26 \pm 0.18$ , respectively) were measured. The relative amplitude of the long

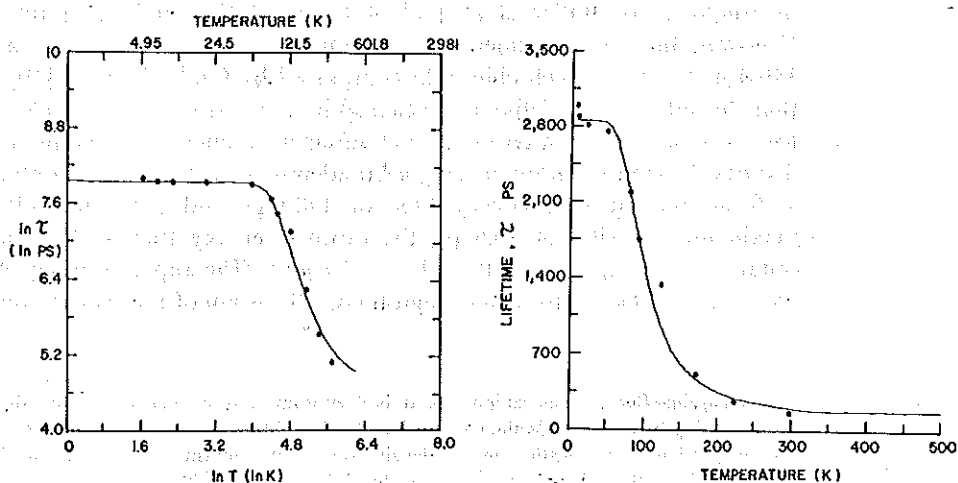


Fig. 3. Temperature dependence of the measured fluorescence lifetimes from spinach leaf for emission wavelengths  $> 720$  nm. The solid line is the last-square fit to the data for the function:  $\tau = A/[1 + BT^{-1/2} \exp(-E_A/kT)]$  where  $A = 2848$  ps,  $B = 1122 K^{1/2}$ , and  $E_A = 0.0417$  eV. Data below 80 K from Avarmaa et al. [9]. (a)  $\ln \tau$  vs.  $\ln T$  and (b)  $\tau$  vs.  $T$ . Typical error bars are less than 10%.

component decreased to  $0.57 \pm 0.07$  at  $I \approx 1.4 \pm 0.4 \times 10^{15}$  photons/cm<sup>2</sup> per pulse (4 measurements), a decrease of 16% was found on the average, while the values of the long and short component decay time decreased to  $1571 \pm 343$  ps and  $210 \pm 69$  ps respectively. The measured fast component lifetime corresponds to a percentage contribution of  $< 10\%$  to the total fluorescence quantum yield. This small contribution of the fast component is indicative of the presence of only a slight exciton annihilation mechanism which becomes significant only at much higher intensities. In this case the non-exponential fluorescence decay profile is not accompanied by a corresponding quenching of the fluorescence quantum yield.

A plot of the temperature dependence of the fluorescence lifetime for a spinach leaf, for wavelengths of observation greater than 720 nm is shown in Fig. 3. These measurements were carried out at an average excitation pulse intensity of  $\approx 1.4 \times 10^{14}$  photons/cm<sup>2</sup> from 300 to 90 K. The salient feature of the data is the increase in the lifetime with a decrease in temperature. For completeness, we have included with our data in Fig. 3, the fluorescence lifetimes measured by Avarmaa et al. [9].

### Discussion

The observed fluorescence decay time of the 685 nm emission was not affected by lowering of the temperature from 300 to 90 K, the decay time remaining  $\sim 227$  ps. These measurements are indicative of a downhill energy transfer from the 685 nm antennae to the reaction center trap of PS II. It is important to note that downhill energy processes should not be affected by lowering of the temperature.

The risetime for the 735 nm component of  $19.6 \pm 3$  ps is in agreement with the early measurements by Yu et al. [10] of  $13 \pm 7$  ps and is also consistent with the measurement of Butler et al. [16] of the resolution limited risetime of  $\leq 50$  ps. However, these measurements are all much shorter than the 735 nm risetime of  $140 \pm 40$  ps for spinach chloroplasts reported by Campillo et al. [19]. It is possible that this latter result [19] may be caused by defocussing of the streak camera image for the two longer wavelength measurements. Such defocussing can lead to a decreased time resolution causing a broadening of the observed risetime.

From the measured decay time of 1400 ps and a risetime (10–90% of the maximum intensity) of 19.6 ps, the intrinsic energy transfer 'feeding' risetime is obtained as  $\tau_{\text{intrinsic rise}} = 19.6/1.94 = 10$  ps\*. The rapid risetime of the 735 nm emission,  $= 19.6 \pm 3$  ps, reveals that the C-705 group of PS I is not excited by energy

\* In analyzing fluorescence emission temporal profiles it is important to note that there exists a relationship between the risetime, defined as the time difference between the 10 and 90% points in the rising portion of the emission and the feeding time of the emitting state which is intricately connected with the decay time of the state. For a long lived state, the 10 to 90% temporal difference of the risetime is twice the intrinsic energy feeding time. For our case, the decay time of 1400 ps and a 10 to 90% time of 19.6 ps requires a factor of 1.94 to obtain the energy transfer feeding time which is given by  $19.6/1.94 = 10$  ps. The factors were obtained from plots of the function  $\exp(-k_1 t) - \exp(-k_2 t)$  for different  $k_1/k_2$  ratios.

transfer from the 685 nm antennae but by energy transfer from either optically excited accessory pigments or by energy transfer from optically excited antennae of PS I. If this were not the case, the observed risetime of the 735 nm component should have been  $\approx 200$  ps, the decay time of the 685 nm antenna. This is also consistent with the apparent lack of intensity dependent variation of the fluorescence quantum yield of the 735 nm emission over the excitation fluence of  $10^{13}$  to  $10^{15}$  photons/cm<sup>2</sup>. If the energy transfer from the 685 nm antennae to the C-705 of PS I were prominent, then the onset of annihilation for PS I would have to be determined by the quenching of the 685 nm antennae. Because of the small size and relatively small absorption cross section of the PS I or C-705 units, annihilation should occur at a much higher intensity in these units than in the 685 nm antennae for direct pumping of the C-705. Using a size and cross-section for the antennae of PS I with a ratio of 100 to 10 for Chl *a*/C-705, the onset of multiple hits and consequently annihilation for direct optical excitation occurs at a photon fluence greater [24] than  $10^{15}$ – $10^{16}$  photons/cm<sup>2</sup>, respectively. These results suggest that the light harvesting antennae do not transfer large amounts of energy to the C-705 group in PS I.

Assuming the presence of a photochemically active trap (P-700) for PS I at a higher energy than the fluorescent 735 nm accessory pigment (C-705), an estimate of the location of the trap energy level may be obtained from an analysis of the temperature dependence of the fluorescence lifetime. The solid curve in Fig. 3 is a plot of  $\tau = A/[1 + BT^{-1/2} \exp(-E_A/RT)]$  (this form arises from Eqns. 1 and 2 discussed below) for an activation energy of 0.042 eV.

In order to extract fundamental information on excitation energy transfer, the temperature dependence of the 735 nm band was analyzed relative to a basic theoretical model. The temperature dependence of the Förster excitation energy transfer rate is given implicitly in terms of the line shape of the fluorescence band of the donor molecule (C-705) and the absorption band of the acceptor molecule (P-700). This calculation however requires knowledge of the absorption and fluorescence line shapes of these molecules for each temperature. Jortner [25,26], Yomosa [27], Sarai [28], and Sarai and Yomosa [29] have described the temperature dependence of excitation energy transfer between the donor and acceptor molecules in terms of an adiabatic phonon-electron coupling. Using this model they have obtained an explicit functional expression for the excitation energy transfer rate as a function of temperature. An important feature of this theory is the presence of a continuous transition from an activationless transfer rate at low temperature to an active rate expression at high temperature. The activationless region arises from tunneling between the nuclear potential surfaces. The excitation energy transfer rates [25,27] between the donor and acceptor molecules for the high temperature case is given by:

$$k_{HT} = \frac{A}{kT^{1/2}} \exp \left[ -\frac{(\Delta E - E_m)^2}{4E_m kT} \right] \quad (1)$$

and for the low temperature case is given by:

$$k_{LT} = \frac{\sqrt{2}A}{(\langle \hbar\omega \rangle)^{1/2}} \exp \left[ -\frac{(\Delta E - E_m)^2}{2E_m \langle \hbar\omega \rangle} \right] \quad (2)$$

where the activation energy  $E_A$  is defined by  $(\Delta E - E_m)^2/4E_m$ ,  $E_m$  is the electron-phonon coupling energy [26]  $\approx S \langle \hbar\omega \rangle$ , with  $S$  the electron-phonon coupling strength, and  $\Delta E$  is the energy gap between the initial and final state nuclear coordinates. The onset between these two temperature regions occurs in the temperature range [26] given by  $kT/\langle \hbar\omega \rangle \sim 0.1-0.2$ , where  $\langle \hbar\omega \rangle$  is the average energy of the phonons involved in the transfer process. A plot of  $\log \tau$  vs.  $\log T$  demonstrates the characteristic behavior of the multiphonon excitation energy transfer occurring throughout this temperature range. This procedure was used by DeVault and Chance [30] in analyzing the temperature dependence of the fluorescence lifetime rather than the conventional Arrhenius plot of  $\tau$  vs.  $T$ . In Fig. 3a, our data have also been plotted in this manner. For completeness we have included in Fig. 3 the fluorescence lifetimes measured by Avarmaa et al., [9] for temperatures below 80 K and a plot of  $\tau$  vs.  $T$ . The data displayed in Fig. 3 for energy transfer from C-705 to P-700 show the characteristic behavior of a continuous transition from an activated to an activationless process as the temperature is lowered. However, it is not possible to fit the entire curve to an Arrhenius dependence. These data are suggestive of the multiphonon theory of excitation energy transfer in the photosynthetic system proposed by Yomosa [27]. From Fig. 3a, the onset temperature between activated and activationless regions is  $\sim 90$  K, which yields the active phonon energy [26,27]  $\langle \hbar\omega \rangle \sim 418 \text{ cm}^{-1} = 0.052 \text{ eV}$ . From Fig. 3, the activation barrier energy is determined to be  $E_A \sim 336 \text{ cm}^{-1} = 0.0417 \text{ eV}$ . The energy gap between the nuclear potential surfaces of C-705 and P-700 molecules is  $\Delta E = 0.0126 \text{ eV}$ . From Eqn. 1,  $E_A = (\Delta E - E_m)^2/4E_m$  and substituting in  $\Delta E$ , one calculates  $E_m = 0.191 \text{ eV}$ . This gives an intermediate electron-phonon coupling strength of  $S = 3.7$  calculated from the relationship [26,27]  $E_m \approx S \langle \hbar\omega \rangle$ .

In conclusion, the temperature dependence of the fluorescence decay kinetics and the low temperature risetime of the 735 nm emission from a spinach leaf have been measured. The data have been interpreted in terms of an electron phonon theory in order to give new insight into the interaction coupling strengths governing the energy transfer mechanism in the primary energy transfer process in photosynthesis.

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In the second section, the author outlines the various methods used to collect and analyze the data. This includes both manual and automated processes. The goal is to ensure that the data is as accurate and reliable as possible.

The third part of the document provides a detailed breakdown of the results. It shows that there has been a significant increase in sales over the period covered. This is attributed to several factors, including improved marketing strategies and better customer service.

Finally, the document concludes with a series of recommendations for future actions. These include continuing to invest in marketing, improving operational efficiency, and maintaining a strong focus on customer satisfaction.

The data shows a clear upward trend in sales, which is a positive indicator for the company's growth. It is essential to continue monitoring these trends and adjust strategies accordingly to stay competitive in the market.