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THE RELATIONSHIP BETWEEN THE LIFETIME AND YIELD OF THE 735 nm FLUORESCENCE OF CHLOROPLASTS AT LOW TEMPERATURES

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Summary

The lifetime and relative yield of the 735 nm fluorescence of chloroplasts, over a range of low temperatures (—60 to —196°C) where the yield of fluorescence changes markedly, were found to be directly proportional. It is concluded that the species of chlorophyll responsible for the 735 nm fluorescence, C-705, is present over the entire temperature range but is less fluorescent at the higher temperatures because of greater energy transfer to *P*-700. It is also concluded from attempts to measure the rise-time of the 735 nm fluorescence at —196°C that the rise-time is less than 50 ps.

Introduction

The 735 nm emission band which is prominent in the emission spectrum of chloroplasts at low temperature has been shown to be a fluorescence emission from Photosystem I and, as such, has been used to monitor excitation energy in Photosystem I [1]. This fluorescence, however, is not simply an emission from the bulk of the antenna chlorophyll in Photosystem I; rather it appears to originate from energy trapping centers in that antenna chlorophyll. It is important to define the characteristics of the low temperature 735 nm emission in relation to other energy utilizing and dissipating processes in Phototsystem I if that fluorescence is to be used properly as an analytical tool. The purpose of the present study was to investigate the mechanism responsible for the strong temperature dependence of the 735 nm fluorescence emission.

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Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

The 735 nm emission band was shown by fluorescence excitation spectroscopy to be due to small amounts of a 705 nm absorbing species of chlorophyll, C-705, which traps excitation energy in the antenna chlorophyll of Photosystem I [2]. It was expected that such traps should function in competition with *P*-700 for the excitation energy in Photosystem I and, indeed, recent experiments comparing the relative yield of the 735 nm fluorescence with the rate of photooxidation of *P*-700 over a range of low temperatures (—78 to —196°C) confirmed that those two processes were in competition with one another [3]. It was proposed that either C-705 only formed on cooling to low temperatures (as an aggregate might form), in which case the temperature dependence of the 735 nm fluorescence would be the temperature dependence for the formation of C-705, or that C-705 was present over the entire temperature range but only became fluorescent at low temperatures. In the latter case lowering the temperature might cause the absorption maximum of C-705 to shift to longer wavelength thereby decreasing the probability for energy transfer from C-705 to *P*-700 with a concomitant increase in the yield of fluorescence from C-705.

The present study was undertaken to distinguish between those two possibilities. If C-705 only forms on cooling, the yield of the 735 nm fluorescence should depend on the concentration of C-705 present at any given temperature but the lifetime of that fluorescence should be constant and independent of the yield. On the other hand, if C-705 is present over the entire temperature range but becomes more fluorescent at lower temperatures because of a greater probability for deexcitation via fluorescence, the lifetime of the 735 nm fluorescence should be proportional to its yield.

Previous comparisons of fluorescence lifetimes of chloroplasts [4] and of Photosystem I subchloroplast fragments [5] at room temperature and at liquid nitrogen temperature found much shorter lifetimes at room temperature but it is not clear in those experiments that the same fluorescing species was involved at both temperatures. In chloroplasts at room temperature where the yield of Photosystem I fluorescence is very low, the fluorescence in the 730 nm region is dominated by the long wavelength tail of the emisssion from Photosystem II while in Photosystem I fragments the emission is at 682 nm at room temperature and 735 nm at —196°C [5,6]. The present study was confined to a temperature range where the 735 nm fluorescence was always present as a well-defined emission band but of variable intensity depending on the temperature of the sample. The measurements show clearly that the lifetime of the 735 nm fluorescence is directly proportional to the yield of fluorescence.

Materials and Methods

Chloroplasts were isolated from pea leaves by homogenizing in a solution containing 0.33 M sorbitol, 50 mM Tris-HCl buffer (pH 7.8) and 20 mM NaCl. After filtering through filtering silk, intact chloroplasts were isolated by centrifugation at 3000 × *g* for 1 min. The chloroplast pellet was resuspended in a medium containing 0.33 M sorbitol and 10 mM Hepes buffer (adjusted to pH 7.6 with 2 M Tris base). Prior to freezing to liquid nitrogen temperature, an aliquot of chloroplasts was diluted into distilled water and an equal volume of

0.66 M sorbitol and 20 mM Hepes buffer (pH 7.6) added. The final chlorophyll concentration in the diluted suspension was 200 μg per ml.

The fluorescence lifetimes of the 735 nm emission from the samples were recorded with a picosecond laser-streak camera system which has been described in detail elsewhere [7—9]. A single 6 ps (full width at half-maximum height) 530 nm excitation pulse was used throughout these measurements. There was some variation in intensity between light pulses ($1—5 \cdot 10^{14}$ photons/cm^2 at the sample) but most data were taken at an intensity of $2 \cdot 10^{14}$ photons/cm^2. No intensity-dependent variation of the lifetime of the 735 nm fluorescence was observed over this range of intensities. Fluorescence emitted by the sample was passed through a Schott RG715 cut-off filter (79% transmission at 730 nm; 1.1% transmission at 690 nm) and focused onto the slit of an S20 IMACON 600 streak camera (John Hadland Ltd.) with a optical multichannel analyzer digital readout. Data were transferred to a computer punch tape for analysis. The fluorescence lifetimes were recorded at a streak speed giving 100 ps time resolution and a full scale coverage of 10 ns; rise-time measurements at —196°C were made with a time resolution of 50 ps and a full scale coverage of 2 ns.

Fluorescence quantum yield measurements were performed with the optical arrangement described by Porter et al. [7] which uses a small portion of the excitation pulse as a reference to the excitation intensity. Typical traces from this system are shown in Fig. 1. The relative quantum yield was obtained by taking the ratio of the area under the fluorescence decay curve with respect to that under the reference pulse using the optical multichannel analyzer to integrate the function.

The samples were contained in a 1-mm diameter phosphorescence tube mounted in a phosphorescence dewar at —196°C. Fluorescence quantum yield and lifetime measurements were performed at various temperatures as the sample was warmed from —196°C to approx. —60°C. A copper-constantin thermocouple embedded in the sample was used to monitor the temperature with a digital voltmeter, the reference junction being maintained at —196°C.

Results

The fluorescence ($\lambda > 715$ nm) from chloroplasts due to excitation by a single 6-ps light pulse at 530 nm of intensity about $2 \cdot 10^{14}$ photons/cm^2 is shown in Fig. 1 for three different temperatures (—196, —150 and —100°C). A reference pulse indicating the relative intensity of the pulse which excited fluorescence is shown on each trace in advance of the fluorescence signal, as described in ref. 7. The width of the light pulse signal also indicates the time resolution of the measurement which is determined by the streak camera speed. It is apparent from the curves in Fig. 1 that the lifetime of the 735 nm fluorescence is shorter at the higher temperatures.

At each temperature examined, the 735 nm fluorescence decayed as a single exponential so that the lifetime of fluorescence was obtained from the reciprocal of the first-order rate constant for decay. The relative yield of fluorescence was determined by dividing the area under the fluorescence curve by the area under the light pulse curve as described previously [7]. The plot of life-

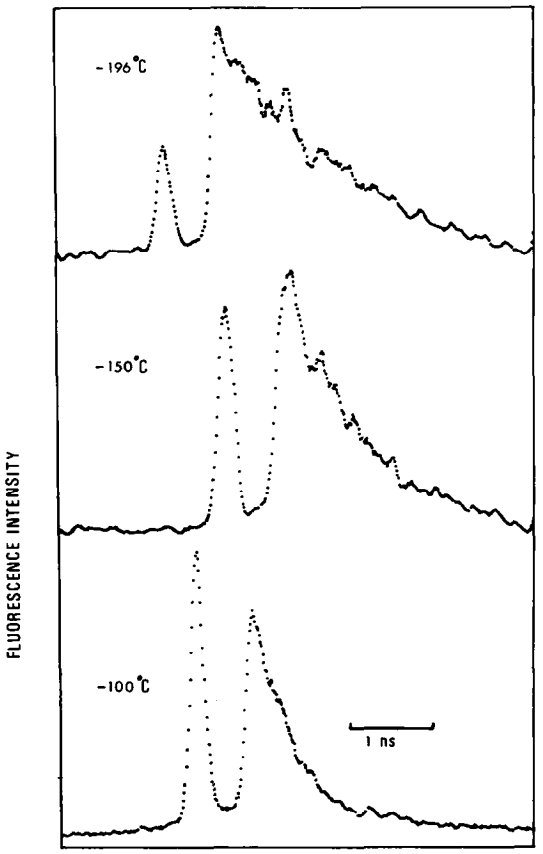

Fig. 1. Kinetics of the 735 nm fluorescence emission from chloroplasts as a function of temperature. Fluorescence was excited with a single pulse (6 ps) of 530 nm light as described in Materials and Methods. The curve preceding the fluorescence signal is the reference pulse used to estimate the relative quantum yield. The excitation intensity was $2 \cdot 10^{14}$ photons/cm^2.

time versus yield (Fig. 2) shows that the lifetime is directly proportional to the yield over the range of temperature investigated (—60 to —196°C).

The data shown in Fig. 2 were obtained from a single frozen sample which was taken through several cycles of warming and recooling with liquid nitrogen. Because the same sample was used for the entire temperature profile and therefore received multiple flashes, these measurements may be considered to be done at the F_M level. The value of the lifetime of the fluorescence obtained at -196°C was approx. 2 ns although a lifetime as long as 2.9 ns was obtained after warming and recooling of the sample to —196°C.

Attempts were also made, with the streak camera set for greater time resolution, to confirm the results of Campillo et al. [10] showing that the rise-time of the 735 nm fluorescence at —196°C was about 140 ps. A typical experiment using the same intensity of excitation as used by Campillo et al. [10] is shown in Fig. 3. The rise-time indicated is approx. 50 ps but it is apparent from the half-width of the pulse trace (shown in the solid line) that the rise-time is limited by the time resolution of the streak camera. The data in Fig. 3 were obtained on a sample that had been preilluminated to obtain the F_M level but

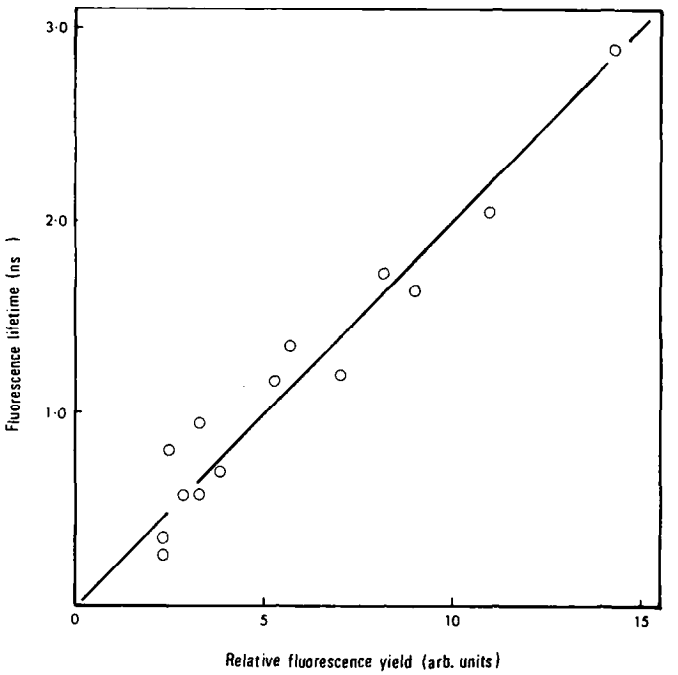

Fig. 2. The yield and lifetime relationship of the 735 nm emission. The data were calculated from the experiment shown in Fig. 1 where the yield was varied by changing the temperature of the sample between —60 and —196°C.

similar results were obtained with a dark-adapted sample (at the F_O level). We conclude from a number of such experiments that the rise-time of the 735 nm fluorescence at —196°C is less than 50 ps.

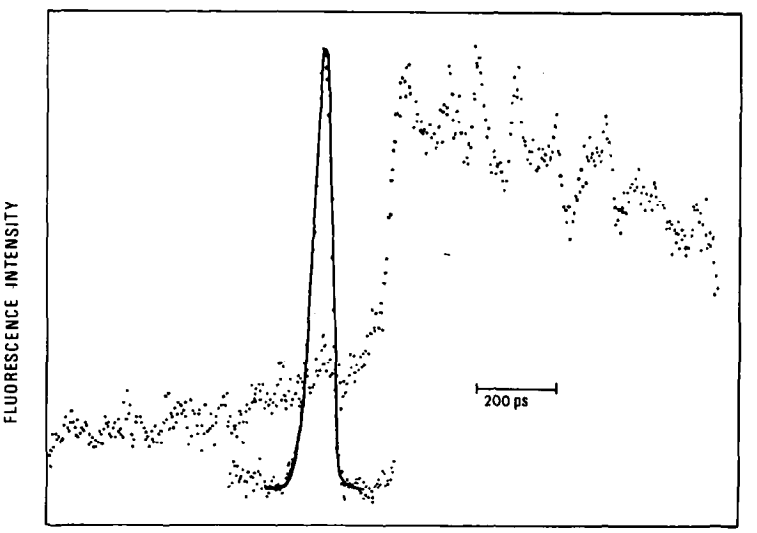

Fig. 3. Measurement of the rise-time of the fluorescence emission at 735 nm at —196°C. Rise-time measurements were made at the F_M level in a sample which had been preilluminated for 2 min with white light at —196°C. The solid line indicates the pulse trace under the conditions used while the dotted curve indicates the measurement of the fluorescence signal under comparable conditions.

Discussion

The results establish that the lifetime of the 735 nm fluorescence is proportional to the yield of fluorescence over a range of low temperatures where the yield changes markedly. We conclude that C-705 is present at the higher temperatures but has a lower fluorescence yield because the excitation energy in C-705 can be transferred on to *P*-700. On lowering the temperature, energy transfer out of C-705 becomes more difficult and the yield of fluorescence from C-705 increases. It is possible that at physiological temperatures C-705 functions as a long wavelength form of chlorophyll which serves to direct the transfer of excitation energy from the antenna chlorophyll of Photosystem I to *P*-700 as was suggested previously in the model of Seeley [11].

The data on the rise-time of the 735 nm fluorescence indicate that excitation energy spreads rapidly throughout Photosystem I and that the diffusion time for excitons to reach the C-705 trapping centers is less than 50 ps. Measurements of the rise-time of the 735 nm fluorescence were made at the F_0 level of dark-adapted chloroplasts but the kinetics were the same as those shown in Fig. 3 for the F_M level of preilluminated chloroplasts. We had thought that the rise-time to the F_M level might show a slower component due to energy transfer from Photosystem II to Photosystem I since such energy transfer contributes more to the F_M level than to the F_0 level [12]. At the pulse intensities used ($2 \cdot 10^{14}$ photons/cm^2) some singlet-singlet annihilation may occur in Photosystem II which would decrease the lifetime of the excitation energy in Photosystem II and, therefore, any rise-time for Photosystem I fluorescence due to energy transfer from Phototsystem II. However, the correlation of fluorescence yield and lifetime using pulse intensities of the magnitude does not indicate significant exciton annihilation (see Fig. 2 and ref. 7). Measurements at greater time resolution are needed to resolve the question of the rise-time of the 735 nm fluorescence.

Note added in proof (Received October 6th, 1978)

Since submitting this manuscript, we have also found that the lifetime of the 735 nm fluorescence was the same at both the F_m and F_o levels at −196°C even though the yield of fluorescence was approximately 40% greater at the F_m level.

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