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PICOSECOND LASER STUDY OF FLUORESCENCE LIFETIMES IN SPINACH CHLOROPLAST PHOTOSYSTEM I AND PHOTOSYSTEM II PREPARATIONS

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SUMMARY

Fractions enriched in either Photosystem I or Photosystem II have been prepared from chloroplasts with digitonin. A more detailed analysis of the decay kinetics of fluorescence excited by a picosecond laser pulse has been possible compared to experiments with unfractionated systems. The Photosystem I fractions show a very short component ($\leqslant$ 100 ps) at room temperature which is apparently independent of pulse intensity over the range of photon densities used ($5 \cdot 10^{13}$–$1 \cdot 10^{16}$ photons cm^{-2}). The Photosystem II fraction has a short initial lifetime at room temperature which is strongly intensity-dependent approaching 500 ps at low photon densities, but decreasing to close to 150 ps at the highest photon densities. All of these room temperature decays appear to be non-exponential, and may possibly be fitted by at $t^{\frac{1}{2}}$ expression, expected from a random diffusion of excitations via Förster energy transfer. On cooling to 77 K, lifetimes of both Photosystem I and Photosystem II increase, the lengthening with Photosystem I being more striking. The Photosystem I decays become intensity dependent like the Photosystem II, and at the lowest photon densities decays which are more nearly exponential within the experimental error give initial lifetimes of about 2 ns. The non-exponential decays seen at high photon densities appear to fit a $t^{\frac{1}{2}}$ expression.

INTRODUCTION

Measurement of in vivo chlorophyll fluorescence lifetimes can give valuable information about the mechanisms of energy transfer and trapping in photosynthesis. Recent studies have identified fluorescence decays at times greater than 100 ps after the excitation pulse which seem to correlate with the concept of a light harvesting array with trapping at randomly distributed sites [1]. The longer lifetimes are almost cer-

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tainly associated with chlorophylls which can transfer their energy to the traps of Photosystem II. The expected increase in intensity of the longer components of the decay was indeed observed when the Photosystem II traps were closed [1, 2].

However, unlike Photosystem II, those chlorophylls which transfer their energy to the Photosystem I (PS1) traps have a very low fluorescence yield and presumably correspondingly short lifetimes. As yet, it has not been possible to identify with certainty that part of the fluorescence decay which represents the chlorophylls of Photosystem I. Short-lived components have been observed by several groups [1–5] but their origin is not clear. This communication describes studies designed to measure lifetimes of the Photosystem I and Photosystem II chlorophylls in different types of sub-chloroplast preparations, characterised by their absorption and emission spectra. The accompanying report (Porter, G., Synowiec, J. A., and Tredwell, C. J.) describes effects of laser pulse intensity on lifetimes in *Chlorella.*

MATERIALS AND METHODS

Chloroplasts were isolated from spinach following the procedure previously described [6] and used to prepare three types of fractions.

(*i*) *Stromal lamellae vesicle fraction.* Chloroplasts were resuspended in 0.2 % (w/v) digitonin (Sigma) in TEM buffer pH 8.0 (50 mM Tris · HCl pH 8.0, 2 mM Na_2EDTA and 5 mM $MgCl_2$) to give a final ratio of digitonin: chlorophyll of 1 : 1 (by wt.) [7]. After 30 min, the suspension was centrifuged at 10 000 × *g* for 30 min. The supernatant was then centrifuged at 80 000 × *g* for 60 min to give a green pellet. This stromal lamellae vesicle pellet was resuspended in 0.2 % digitonin in TEM buffer.

(*ii*) *F_I fraction.* Chloroplasts were incubated with 1.3 % digitonin (w/v) at a digitonin : chlorophyll ratio of 10 : 1 in TEM buffer for 60 min, and centrifuged at 80 000 × *g* for 60 min to give a clear green supernatant. This was layered on a 10–30 % (w/w) sucrose density gradient in TEM buffer containing 0.5 % digitonin (w/v), and the system allowed to reach equilibrium by centrifugation in a swing-out rotor at 40 000 × *g* for 64 h [7]. The most dense green band, representing F_I, was removed with a syringe and concentrated after dilution with an equal volume of TEM buffer by centrifugation at 400 000 × *g* for 60 min in the Titanium 10 × 10 ml rotor of a M.S.E. Superspeed 65. The F_I pellet was resuspended in 0.2 % digitonin in TEM buffer.

(*iii*) *Digitonin-extracted-fragment fraction.* The 80 000 × *g* precipitate of chloroplast fragments from the F_I fraction preparation was resuspended in 1.3 % digitonin (w/v) in 2.0 % (w/v) NaCl and 50 mM Tris · HCl pH 8.0 to the same volume as the chloroplast suspension during the initial digitonin treatment. After 60 min, the suspension was centrifuged at 30 000 × *g* for 30 min and the supernatant discarded. The extraction of the fragments with 1.3 % digitonin was repeated 3–4 times until the supernatant was only pale green in colour. The fragment pellet was resuspended in 1.3 % digitonin once more, centrifuged at 3000 × *g* for 4 min to remove large fragments, and then centrifuged at 30 000 × *g* for 30 min to give a pellet. This was resuspended in 0.2 % digitonin in TEM to give a clear non-scattering solution. Chlorophyll concentrations were measured according to Arnon [8], but using the modified factor of Bruinsma [9] for total chlorophyll.

Absorption spectra were measured at room temperature on a Unicam SP800

scanning spectrophotometer, and the wavelength scale was checked with holmium or didymium filters. The cytochrome content of the digitonin-extracted fragment fraction was determined from oxidised-reduced difference spectra on an Aminco Chance DW2 Spectrophotometer. The molar absorption of cytochrome *b* 559 was taken to be 21 000 at 559 nm [10].

Fluorescence emission spectra were obtained on a Perkin-Elmer MPF4 fluorescence spectrophotometer and corrected for photomultiplier sensitivity, but not for monochromator response. At room temperature, samples were placed in a 10×10 mm cuvette and at 77 K in cylindrical spectrosil tubes of internal diameter 2.0–2.5 mm. Samples were diluted with buffer (10 mM Tris · HCl, pH 8.0), until the shape of the emission spectrum no longer altered on further dilution, only then could distortion due to self-absorption be discounted. At room temperature the excitation wavelength was 440 nm; but at 77 K 380 nm was used with a 430 nm filter in front of the photomultiplier to reduce effects of scattered light. The emitted light was observed at right angles to the excitation beam.

The steady state fluorescence yield of the digitonin-extracted fragment fraction was measured on illumination with broad-band blue-green light (Schott BG18, 4 mm and Schott BG38, 2 mm), intensity about 50 W/m^2. The fluorescence emitted at right angles was passed through a Schott RG 665 cut-off and Balzer B40 685 nm interference filter on to the photomultiplier.

The fluorescence lifetimes were measured on the picosecond laser apparatus described earlier [2]. Fluorescence was excited by a laser pulse, wavelength 530 nm, width at half-height 6 ps. The photon density of the beam was measured with an I.T.L. laser calorimeter, and was varied between $5 \cdot 10^{13}$ and $10^{16} \pm 25\%$ photons cm^{-2}.

The full pulse train was not used, but a single pulse was selected out of the train with an electro-optic device incorporating a Pockels cell. The concentration of the samples was in the range 0.2–0.6 mg chlorophyll ml^{-1}, with transmission at 530 nm in the range 30–80 %. The samples were placed in a 1 mm pathlength cuvette for room temperature study and in a cylindrical tube (internal diameter 1 mm) immersed in liquid nitrogen in an optical dewar for study at 77 K. The samples were kept dark both before and between successive measurements. A period of several minutes was allowed between each shot to allow return to the dark-adapted state for the room temperature samples.

Fluorescence emitted co-axially by the sample was passed through a selection filter on to the entrance slit of an Imacon 600 streak camera with a photocathode having S20 sensitivity. The streak intensity was observed with a vidicon multichannel analyser, stored in a 500 channel memory, and finally presented as a plot of fluorescence intensity against time on a flatbed XY recorder.

RESULTS

In Fig. 1, the absorption and fluorescence emission spectra for stromal lamellae vesicle fraction and F_I preparations are shown. The stromal lamellae vesicle fraction shows absorption maxima at 680 and 437 nm, the same as that of chlorophyll *a* in untreated chloroplasts. Small amounts of carotenoid are present as indicated by the absorption shoulders at 470 and 490 nm. At room temperature, the very weak emis-

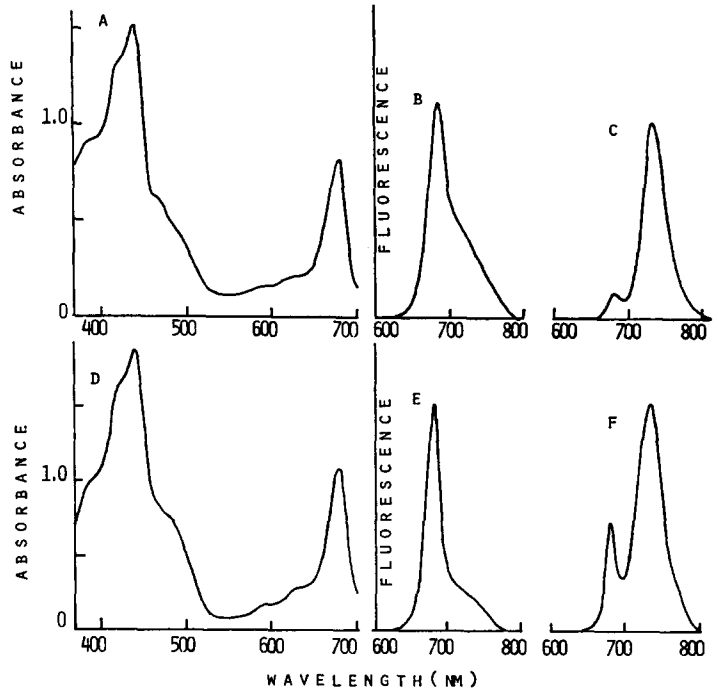

Fig. 1. The absorption and fluorescence emission spectra of the Photosystem I fractions, stromal lamellae vesicle fraction: (upper, A-C) and F_1 (lower, D-F). (A) and (D) are absorption spectra at room temperature, (B) and (E) the fluorescence emission spectra at room temperature, and (C) and (F) the fluorescence emission spectra at 77 K. The fluorescence emission spectra are corrected for photomultiplier (Hamamatsu R446) spectral response. The scales are in arbitrary units and are not comparable.

sion is at 682 nm, with a long wavelength tail centred at 730–740 nm. At 77 K however, the major emission band is at 730 nm with only relatively weak emission at 682 nm. The latter cannot be ascribed to solubilised chlorophyll as this emits maximally at 675 nm, at both temperatures.

In the case of the F_1 preparation, the absorption maxima are shifted 1 nm to the blue (679 and 436 nm) indicating perhaps a very slight change in micro-environment compared to in vivo chlorophyll *a*. As found with the stromal lamellae vesicle fraction, the spectrum also shows weak shoulders at 470 and 490 nm. At room temperature an emission peak at 681 nm is seen together with a long wavelength tail, centred at 730–740 nm. At 77 K, two emission peaks are observed, at 682 and 728 nm, contrasting with a single peak at 730 nm reported by Wessels and Borchert for the same type of preparation [11].

In Fig. 2, the absorption and fluorescence emission spectra for the digitonin-extracted fragment fraction preparations are shown. The absorption spectrum is characterised by the chlorophyll *b* bands at 653 and 471 nm. The chlorophyll *a* maxima at 680 and 436 nm are little changed from untreated chloroplasts. The fluorescence emission spectrum at room temperature shows a peak at 682 nm with a minor band at 730–740 nm, and on cooling to 77 K the characteristic twin Photosystem II peaks at 683–684 nm and 695–696 nm are observed together with a long

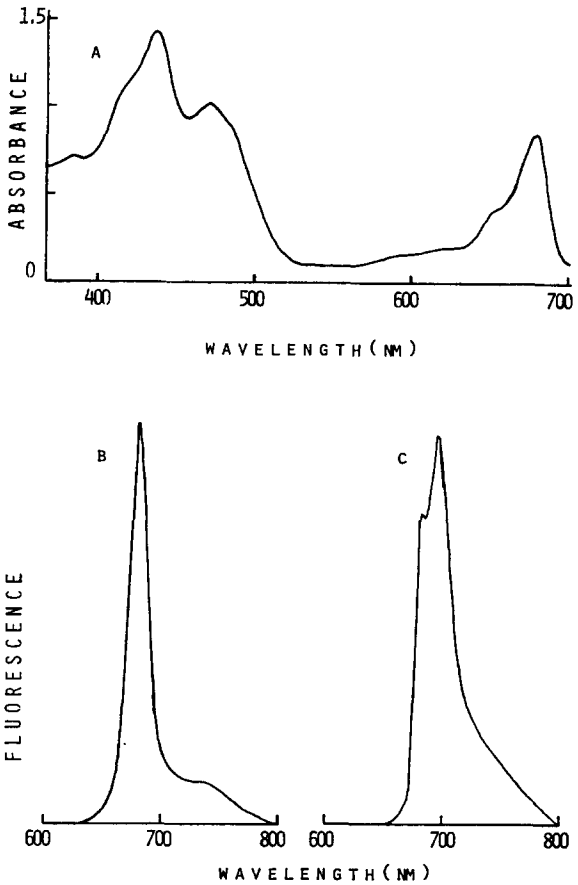

Fig. 2. The absorption and fluorescence emission spectra of the Photosystem II fraction (digitonin-extracted fragment fraction). (A) is the absorption spectrum at room temperature, and (B) and (C) the fluorescence emission spectra at room temperature and 77 K respectively. Other conditions are as in Fig. 1.

wavelength tail. The major 730 nm band seen in chloroplasts, and attributable to Photosystem I, is absent. The ratio of chlorophyll *a* to chlorophyll *b* is found to be 1.76. The only cytochrome detectable in the digitonin-extracted fragment fraction is cytochrome *b* 559, a characteristic component of Photosystem II, present in the proportion of 1 mol per 267 mol total chlorophyll.

As the state of the photochemical traps has been found to influence the Photosystem II fluorescence lifetime [1], the steady state fluorescence yield of the digitonin-extracted fragment fraction was investigated to attempt to define the state of the traps. The digitonin-extracted fragment fraction in 10 mM Tris · HCl pH 8.0 buffer shows a slight progressive decrease in fluorescence yield on continuous illumination, which recovers in the dark. Addition of dithionite does not increase the fluorescence yield but it is however stable during illumination. The effect of 20 μM DCMU is similar. As electron donation from water is almost certainly inhibited during the preparation, 10 mM NH_2OH was added, but still no light-induced increase in fluorescence yield

could be seen. Although it would therefore appear that the photochemical traps are closed, care should be exercised in the interpretation of these observations, as during the preparation of the digitonin-extracted fragment fraction both secondary electron donors and secondary electron acceptors of Photosystem II are probably lost, artificial cyclic pathways around Photosystem II may be induced or the back reaction promoted. Indeed, at 77 K, dithionite is found to produce the increase in fluorescence yield expected if the traps were open in untreated digitonin-extracted fragment fraction. We suggest that the traps in digitonin-extracted fragment fraction are in fact predominantly open at room temperature, the lifetime measurements reported below supporting this. But at 77 K they may be closed when dithionite is added.

In Fig. 3, examples of the room temperature fluorescence decay curves for these preparations are shown. The fluorescence lifetime measurements on Photosystem I fractions at room temperature are not as precise as those on Photosystem II as the low fluorescence yield leads to low signal to noise ratios at short time resolutions. As the output from our multichannel analyser is not computer compatible, signal averaging techniques cannot conveniently be used at present. The usefulness of signal averaging to improve the signal to noise ratio would anyway be reduced by the inherent variability from shot to shot of the laser pulse intensity. The decay curves were analysed by plotting the logarithm of the fluorescence intensity at short time intervals along the curve either against time (t) or the square root of time ($t^{\frac{1}{2}}$). The fluorescence maximum

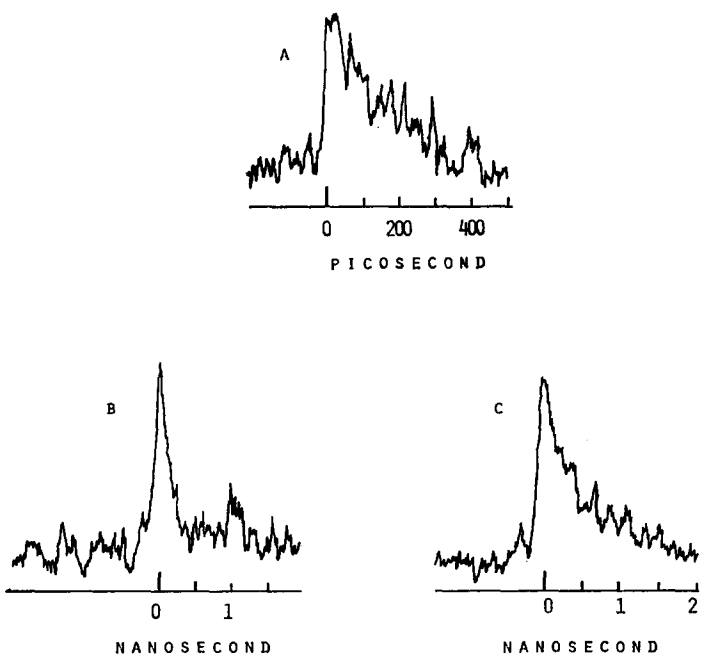

Fig. 3. The room temperature fluorescence decay of Photosystems I and II. (A) shows the decay of stromal lamellae vesicle fraction fluorescence measured at a short time resolution and a photon density of about $5 \cdot 10^{15}$ photons cm^{-2}. (B) and (C) show decay curves for Photosystem I (stromal lamellae vesicle fraction) and Photosystem II (digitonin-extracted fragment fraction) respectively. (B) and (C) were taken at the same time resolution and at the same low intensity of about $5 \cdot 10^{13}$ photons cm^{-2}.

TABLE I

FLUORESCENCE LIFETIMES IN PHOTOSYSTEM-I AND PHOTOSYSTEM-II ENRICHED FRACTIONS

For room temperature measurements of fluorescence lifetimes on the picosecond laser apparatus, a Schott RG 665 cut-off filter was placed between the sample and the entrance slit of the streak camera. For 77 K experiments with F_I, a Schott RG N9 cut off filter (50 % transmission at 720 nm) was used, and for the digitonin-extracted fragment fraction (DEF) at 77 K the Schott RG 665. For the stromal lamellae vesicle fraction (SLV), at 77 K, either filter gave similar results. The lifetimes quoted were calculated from decay curves obtained at the lowest laser intensities (less than 10^{14} photons cm^{-2}) and which appeared exponential within the experimental error. The number in parentheses indicates the number of values determined. The $t_{1/e}$ values are given for low photon densities for those samples showing non-exponential decay curves even at the lowest intensities used. The value of $t_{1/e}$ is the time taken for the fluorescence to decay to 1/e of its maximum value. The value of the constant A was calculated from the slope of the plot of logarithm of fluorescence intensity against the square root of time for decay curves taken at the highest photon densities used ($5 \cdot 10^{15}$–$1 \cdot 10^{16}$ photons cm^{-2}).

Preparation	Temperature (K)	A ($ps^{-\frac{1}{2}}$)	$t_{1/e}$ (ns)	Lifetime (ns)	
				Range	Average
SLV	293	–	0.1	–	–
	77	0.047	–	1.65–1.95(9)	1.82
F_I	293	–	0.1	–	–
	77	–	–	1.88–1.92(2)	1.90
DEF	293	0.119	0.5	–	–
	77	0.048	–	2.42–2.52(2)	2.47

was taken to be zero time. In some cases, spurious noise spikes were superimposed on the random background noise and these were ignored in the analysis.

The stromal lamellae vesicle fraction and F_I show decay curves indistinguishable at the present resolution, and having $t_{1/e}$ (the time taken for the fluorescence to fall to 1/e of its maximum value) about 100 ps (see Table I). When the laser beam intensity was varied between $5 \cdot 10^{13}$ and 10^{16} photons cm^{-2}, the $t_{1/e}$ lifetime of the Photosystem I fractions remained invariant within the experimental error. Some decays may possibly be fitted to a $t^{\frac{1}{2}}$ expression. However, as an appreciable part of the total decay can occur within the time resolution of the instrument, and also the presence of trace amounts of Photosystem II cannot be completely ruled out, it is not possible at present to define the form of the decay precisely.

The Photosystem II fraction, the digitonin-extracted fragment fraction, shows a decay with an initial lifetime distinctly longer than Photosystem I, as can be seen in Fig. 3. In contrast to Photosystem I, the lifetime is strongly dependent upon laser beam intensity. The $t_{1/e}$ found at low intensities ($5 \cdot 10^{13}$–$1 \cdot 10^{14}$ photons cm^{-2}) is close to 500 ps. The form of the decay, although tending to be more exponential the lower the photon density, remains non-exponential even at the lowest photon density used. When high photon densities are used ($5 \cdot 10^{15}$–$1 \cdot 10^{16}$ photons cm^{-2}) the decay curve is found to fit closely a $t^{\frac{1}{2}}$ expression, with $t_{1/e}$ about 150 ps. An expression of the form

$$I_t = I_0 \exp(-kt - At^{\frac{1}{2}})$$

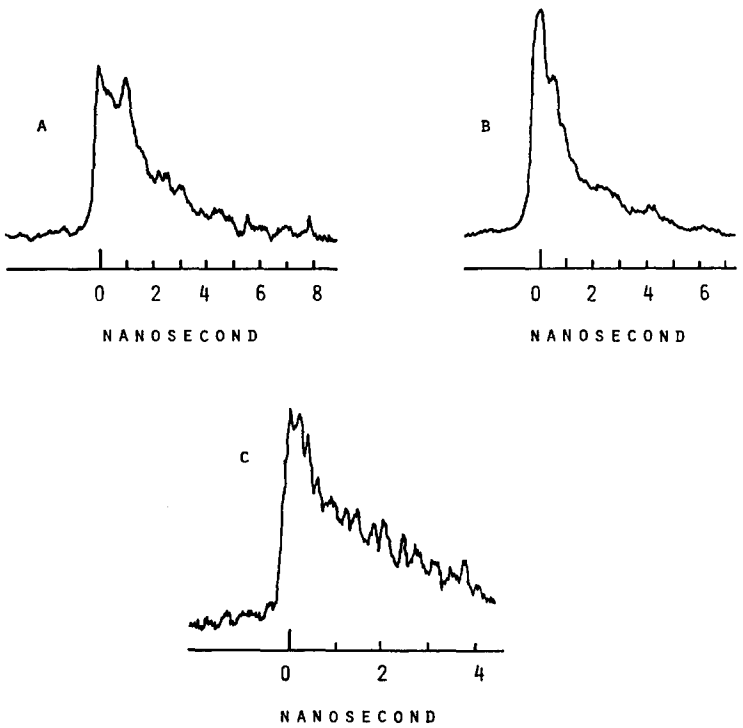

Fig. 4. The fluorescence decay of Photosystem I fractions at 77 K. The effect of photon density on the shape of the decay curve for the stromal lamellae vesicle fraction at 77 K is shown in (A) and (B). (A) is an example of a decay following an exponential within experimental error, and seen at low photon densities ($5 \cdot 10^{13}$–$1 \cdot 10^{14}$ photons cm^{-2}). (B) is an example of a decay on the same time scale which fits closely a $t^{\frac{1}{2}}$ expression and is seen at high photon densities (10^{16} photons cm^{-2}). (C) shows the decay curve of F_I which is found in the range of photon densities from about 10^{14} to 10^{16} photons cm^{-2}, illustrating the two phases seen.

can arise from diffusion of excitons via Förster energy transfer as discussed in ref. 1. From the slope of the $t^{\frac{1}{2}}$ plot, the value for the constant A is found to be 0.12 $ps^{-\frac{1}{2}}$, where A is a function of the concentration of quenching centres and the rate of energy transfer from excited states to quenchers.

Fig. 4 illustrates the striking increase in fluorescence lifetimes seen in Photosystem I fractions on lowering the temperature to 77 K. The decay kinetics for stromal lamellae vesicle fraction are strongly dependent upon laser beam intensity. At low photon densities, the decays are more nearly exponential within the experimental error with an initial lifetime of about 1.9 ns. At higher photon densities the decay curves become non-exponential and can be fitted at the highest intensities used by a $t^{\frac{1}{2}}$ expression, giving a value of the constant A of 0.047 $ps^{-\frac{1}{2}}$. It is found that the latter part of these non-exponential decays can be fitted to an exponential with lifetimes ranging from shot to shot from 1.95 to 2.85 ns. The F_I preparations at 77 K show decays which are much less variable with photon density. Only at the lowest photon density used does the decay appear exponential within experimental error, with an initial lifetime of 1.9 ns. Over the rest of the range of intensities used, the decay is non-

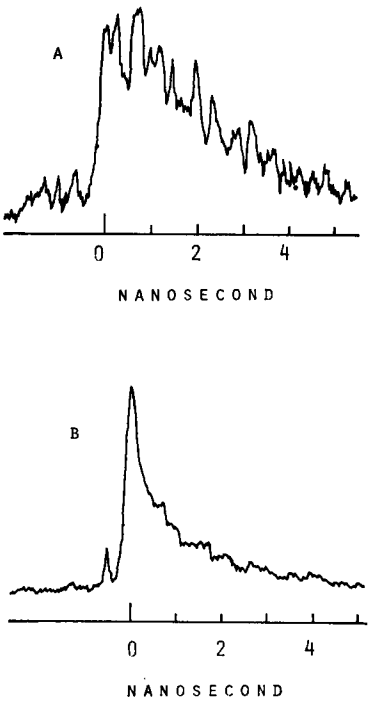

Fig. 5. The effect of photon density on the fluorescence decay of the digitonin-extracted fragment fraction at 77 K. (A) and (B) were taken on the same time scale but at different intensities. In (A) the photon density was low, about $5 \cdot 10^{13}$ photons cm^{-2}, and in (B) the photon density was high, greater than $5 \cdot 10^{15}$ photons cm^{-2}.

exponential and does not fit a $t^{\frac{1}{2}}$ expression even at the highest intensities. Instead it consists of a rapid phase (25–40 % of the total) and a slower phase as shown in Fig. 4, and this second phase can be fitted to an exponential with a lifetime of 2.8 ns. As shown in Fig. 5, the lifetime of Photosystem II also lengthens on cooling to 77 K. The decay curve of the digitonin-extracted fragment fraction at this temperature is strongly dependent on laser pulse intensity. At the lowest photon densities used ($5 \cdot 10^{13}$ photons cm^{-2}), the decay appears exponential within the experimental error and gives an initial lifetime of 2.5 ns. At 10^{14}–$5 \cdot 10^{14}$ photons cm^{-2} the decay becomes non-exponential and initial lifetimes ranging from 2.1 to 0.9 ns are found. At high photon densities ($5 \cdot 10^{15}$ photons cm^{-2}) the decay curve can be closely fitted by a $t^{\frac{1}{2}}$ expression with the value of the constant A of 0.048 $ps^{-\frac{1}{2}}$. These results are summarised in Table I.

Therefore lifetimes of Photosystems I and II at 77 K are comparable both at low and high photon densities.

DISCUSSION

The technique of picosecond laser fluorimetry is relatively new and it is already very clear that care must be taken in interpreting any results obtained by this method. A photon density of 10^{14}–10^{15} photons cm^{-2} is sufficiently high to bring about multi-

ple hitting of photosynthetic units and possibly induce exciton interactions in the light harvesting pigments. Such an effect has already been discussed in recent publications [1, 12–14] and would be expected to be particularly significant in unfractionated systems or in fractions which allow extensive exciton migration. It is for these reasons that previous attempts to use the picosecond laser technique to identify the lifetimes of chlorophylls associated with either Photosystem I or Photosystem II have been open to criticism.

The work presented above does however indicate that picosecond fluorimetry can be used to estimate lifetimes. The considerable increase in lifetime observed when going from 293 to 77 K is in accordance with the fluorescence yield changes which occur under the same conditions. The distinctly longer lifetime of Photosystem II at room temperature compared to Photosystem I, on the same instrument resolution and at the same photon density, is also in line with the known higher fluorescence yield of Photosystem II.

At room temperature, the fluorescence yield of Photosystem I chlorophyll has been reported to be 0.003 [15], corresponding to a lifetime of 57 ps, taking the intrinsic lifetime of chlorophyll *a* as 19 ns [16]. However, this calculation assumes that the fluorescence yield can be equated with the quantum yield, which is probably not the case with 530 nm excitation. At this wavelength, absorption by ground state chlorophyll is low compared to that of carotenoids. However, values of Photosystem I lifetimes in this time region have been reported, $\tau \leqslant 10$ ps [17], $\tau \leqslant 30$ ps [1, 18], $\tau = 60$ ps [4], $\tau = 80$ ps [5] and $\tau = 130$ ps [2]. The measurements reported in this present paper using digitonin prepared vesicles and particles (F_I) do not seem to be intensity dependent over the range of photon densities used, and the estimated initial lifetime of about 100 ps is not incompatible with the reported fluorescence yield for Photosystem I.

The lifetimes of 1.8–2.8 ns measured for Photosystem I fractions at 77 K are also in reasonable agreement with earlier low temperature lifetime measurements on the 730 nm fluorescing component of intact systems [19, 20]. The different behaviour of the digitonin prepared vesicles and F_I at 77 K with variation of the photon density may perhaps be related to their physical size. The recent review by Arntzen and Briantais [21] has discussed the good evidence for Photosystem I only in the stroma lamellae and for the identity of stroma and grana Photosystem I. Both the digitonin prepared vesicles and F_I emit near 680 nm at room temperature, rather than at 730 nm, in line with previously published results for isolated Photosystem I particles [15]. Both the digitonin prepared vesicles and F_I have been characterised by Wessels and coworkers [7, 22] and the digitonin prepared vesicles have been shown to be derived from stroma lamellae possessing Photosystem I but not Photosystem II photochemical activity. The preparation consists of vesicles varying in size from 50 to 200 nm diameter. F_I is derived from both stroma and grana lamellae, possesses only Photosystem I photochemical activity and has a reaction centre concentration of 1 *P*-700 : 120 chlorophyll *a*. F_I particles are much smaller than the digitonin prepared vesicles, being rod shaped with a length of 15 nm and a diameter of 5 nm. It would be expected that multiple photon hitting is less likely to occur in F_I than in stromal lamellae vesicles, and that the effect of high photon densities would be less.

The Photosystem II preparation, the digitonin-extracted fragment fraction, is a residue found after Photosystem I particles are preferentially released from the thyla-

koids by digitonin treatment. It resembles various heavy fractions obtained from chloroplasts by detergent action (see for example ref. 23) and its properties will be fully reported in a further communication. The room temperature lifetime of 500 ps at low intensities is close to that found for intact dark adapted *Chlorella* (see ref. 1 and the accompanying paper). It would appear that the removal of Photosystem I has little effect on the Photosystem II lifetime under the conditions of these experiments.

On cooling to 77 K, the lifetime lengthens to close to the lifetime of Photosystem I at the same temperature. Intact *Chlorella* also shows a similar lifetime at 77 K, 2.5 ns [1] and 2.8 ns (see the accompanying paper). Apparently, exciton migration and trapping in the two photosystems is more comparable at low temperature than at room temperature, where Photosystem I shows a lifetime approximately five times shorter at low intensities.

The form of the room temperature fluorescence decays for Photosystem I and Photosystem II at the lowest intensities measurable is non-exponential and may be consistent with the $t^{\frac{1}{2}}$ relationship. We believe that at the lowest laser pulse intensities the lifetimes measured may reflect the in vivo mechanism of energy migration and trapping in the photosystems.

The isolation of fractions enriched in Photosystem I or II has allowed a clearer analysis of the lifetimes of each photosystem, in particular that of Photosystem I at room temperature. The lifetimes found for Photosystem I at 77 K and for Photosystem II at room temperature and at 77 K agree well with those seen in intact *Chlorella.*

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