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PICOSECOND TIME-RESOLVED FLUORESCENCE STUDY OF CHLOROPHYLL ORGANISATION AND EXCITATION ENERGY DISTRIBUTION IN CHLOROPLASTS FROM WILD-TYPE BARLEY AND A MUTANT LACKING CHLOROPHYLL *b*

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Summary

Picosecond time-resolved fluorescence spectroscopy has been used to investigate the fluorescence emission from wild-type barley chloroplasts and from chloroplasts of the barley mutant, *chlorina* f-2, which lacks the light-harvesting chlorophyll *a/b*-protein complex. Cation-controlled regulation of the distribution of excitation energy was studied in isolated chloroplasts at the F_0 and F_m levels. It was found that:

(a) The fluorescence decay curves were distinctly non-exponential, even at low excitation intensities ($<2 \times 10^{14}$ photons $\cdot$ cm^{-2}).

(b) The fluorescence decay curves could, however, be described by a dual exponential decay law. The wild-type barley chloroplasts gave a short-lived fluorescence component of approximately 140 ps and a long-lived component of 600 ps (F_0) or 1300 ps (F_m) in the presence of Mg^{2+}; in comparison, the mutant barley yielded a short-lived fluorescence component of approx. 50 ps and a long-lived component of 194 ps (F_0) and 424 ps (F_m).

(c) The absence of the light-harvesting chlorophyll *a/b*-protein complex in the mutant results in a low fluorescence quantum yield which is unaffected by the cation composition of the medium.

(d) The fluorescence yield changes seen in steady-state experiments on closing Photosystem II reaction centres (F_m/F_0) or on the addition of $MgCl_2$ ($+Mg^{2+}/-Mg^{2+}$) were in overall agreement with those calculated from the time-resolved fluorescence measurements.

The results suggest that the short-lived fluorescence component is partly attributable to the chlorophyll *a* antenna of Photosystem I, and, in part, to

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Tris, tris(hydroxymethyl)aminomethane; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid.

those light-harvesting-Photosystem II pigment combinations which are strongly coupled to the Photosystem I antenna chlorophyll. The long-lived fluorescence component can be ascribed to the light-harvesting-Photosystem II pigment combinations not coupled with the antenna of Photosystem I. In the case of the mutant, the two components appear to be the separate emissions from the Photosystem I and Photosystem II antenna chlorophylls.

Introduction

It is generally accepted that the thylakoid membranes of higher plants and algae contain three main chlorophyll-protein complexes, namely the light-harvesting chlorophyll *a/b*-protein complex, the Photosystem II antenna chlorophyll *a* protein complex and the Photosystem I antenna chlorophyll *a*-protein complex [1]. The latter two pigment complexes are intimately associated with their respective photochemical reaction centres, whereas the third complex acts as a light-harvesting system which appears to preferentially transfer energy to the Photosystem II antenna complex [2].

For both photosystems to operate efficiently at non-saturating light intensities, there must be an equal distribution of excitation energy between the reaction centres of the two photosystems. In addition to the possibility of direct energy transfer from the Photosystem II antennae to those of Photosystem I [3], there is a mechanism for regulating the amount of excitation energy reaching the Photosystem I antennae from the light-harvesting complex. The latter process can be controlled by the cation composition of the medium surrounding the thylakoid membrane and is commonly known as spillover [4].

Picosecond time-resolved fluorescence spectroscopy can be used to probe the way in which energy migrates between the various pigment-protein complexes of photosynthetic species, as demonstrated by the recent measurements with the red alga, *Porphyridium cruentum* [5,6]. In this paper, we have used the same technique to investigate energy migration between the chlorophyll-protein complexes of higher plants. Our approach has been to study the mutant barley strain, *chlorina* f-2, which lacks the light-harvesting chlorophyll *a/b*-protein complex, and to compare the results with those of wild-type barley as the control. The fluorescence decay kinetics of these two systems have been recorded under various experimental conditions and compared with data obtained from steady-state fluorescence measurements via their relative fluorescence yield changes.

Materials and Methods

Wild-type barley (*Hordeum vulgare*) and the *chlorina* f-2 mutant [7] were grown in soil in a greenhouse under a 16 h light/8 h dark regime. Leaves with a length of 10—20 cm were harvested from the plants when they were several weeks old. Chloroplasts were released from the whole leaf by immersion under low salt buffer (0.33 M sorbitol, 10 mM HEPES with Tris added to pH 7.6) and gently scraping the underside of the leaf with a razor blade. After filtration through 8 layers of muslin and centrifugation at $3000 \times g$ for 2 min, the

chloroplasts were resuspended in a minimum volume of the low salt buffer described above. For steady-state fluorescence yield measurements, the chloroplasts were initially diluted by a factor of about 30 in distilled water (1.5 ml) to break the chloroplast envelope and, after 30 s, 1.5 ml of double strength low salt buffer was added. Further experimental details may be found in the legend to Fig. 3.

Absorption specta were recorded on a scanning spectrophotometer (Unicam SP800). Fluorescence spectra were measured at a sample concentration of 1 μg chlorophyll/ml on a Perkin-Elmer MPF 3 fluorescence spectrophotometer. Chlorophyll concentrations were measured by a method described by Bruinsma [8]; other details are given in the legends to Figs. 1 and 2.

The picosecond laser apparatus used to study the fluorescence decay kinetics has been described in detail elsewhere [9]. Briefly, a Pöckels cell electro-optic shutter selects a single 6 ps (full width at half maximum height) 530 nm pulse from the pulse train generated by a frequency-doubled mode-locked Nd^{3+}:glass laser. Fluorescence emitted by the sample is collected at 180° with respect to the excitation beam and focussed onto the S 20 photocathode of an Imacon 600 streak camera (John Hadland (P.I.) Ltd.). Any residual excitation light is removed by a 665 nm cut-off filter (Schott RG-665) situated before the camera. The fluorescence streak trace is detected by a 500 channel optical multichannel analyser (OMA 1205 A and B, Princeton Applied Research) and stored in a digital memory. The streak rates used in the present work were 100 ps/mm (4.62 ps per OMA channel) and 300 ps/mm (11.6 ps per OMA channel). To avoid exciton annihilation effects, the excitation intensity was kept below $2 \cdot 10^{14}$ photons $\cdot$ cm^{-2}; the transmission of the sample at 530 nm was greater than 70% (1 mm pathlength).

Chloroplast samples at the F_m level were obtained by adding DCMU and preilluminating with a CW He/Ne laser (intensity 12.5 W $\cdot$ m^{-2}) as previously described [10].

Results

Fig. 1 shows the visible absorption spectra of the isolated chloroplasts from both the wild-type and the *chlorina* f-2 mutant barley. The chlorophyll *b* deficiency in the mutant is discernable by the decreased absorption at 650 and 470 nm, the peak at 495 nm is probably due to β-carotene. There are no obvious differences in the 680 nm chlorophyll *a* absorption band of the two strains at the resolution used. At the excitation wavelength, 530 nm, for the kinetic measurements, both strains exhibited approximately equal absorption relative to the maximum at 680 nm.

Fig. 2 shows that the fluorescence emission from the wild-type and the mutant barley are similar at room temperature, although the wild-type chloroplasts exhibit a more pronounced shoulder at 730 nm (Fig. 2b). Upon cooling to 77 K, the differences between the two emission spectra become more apparent; the intensity of the 684 nm emission from the mutant, relative to that of the broad 730 nm band, is considerably lower than that of the wild-type. The 695 nm shoulder, detectable in the emission spectrum of the wild-type barley, is not resolved in the case of the mutant and is possibly obscurred

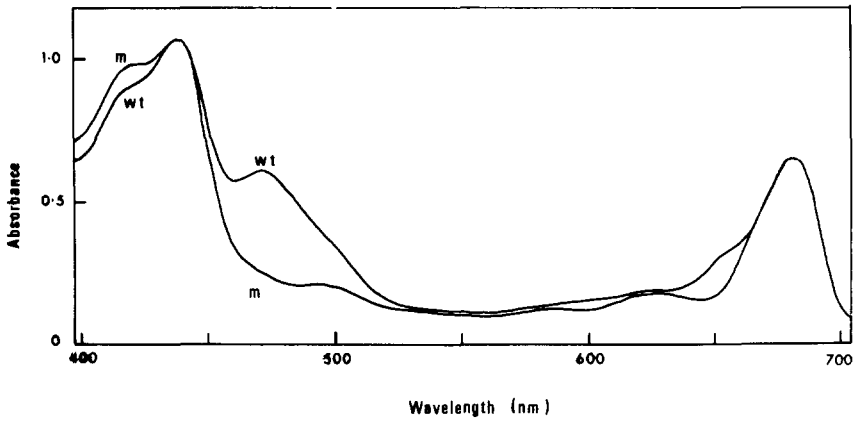

Fig. 1. Absorption spectrum of chloroplasts from wild type and *chlorina* f-2 mutant of barley suspended in low salt buffer. The spectra were taken on a Unicam SP800 spectrophotometer fitted with a diffusing plate to reduce scattering artefacts. Pathlength 10 mm; m, mutant; wt, wild type.

by the intense 730 nm band; the 730 nm band of the mutant is also blue-shifted by approx. 12 nm with respect to that of the wild-type.

The chlorophyll fluorescence induction curves of dark-adapted chloroplasts in the presence of DCMU are shown in Fig. 3. The fluorescence yield of the wild-type barley rises rapidly to a maximum which is almost twice as intense in the presence of Mg^{2+} (curve a) as in its absence (curve b). Mutant chloro-

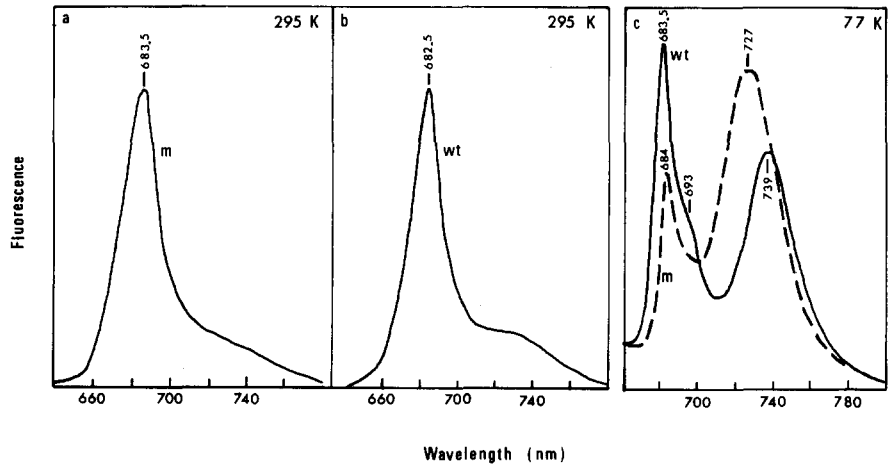

Fig. 2. Fluorescence emission spectra of chloroplasts from wild-type and *chlorina* f-2 mutant of barley. (a) mutant at room temperature; (b) wild-type at room temperature; and (c) mutant (m) and wild type (wt) at 77 K. The spectra were taken on a Perkin-Elmer MPF3 spectrofluorimeter fitted with a Hamamatsu R446S phototube, and not corrected for detector sensitivity. A Schott RG610 cut-off filter was placed between the sample and the phototube. The samples were excited at 436 nm, emission being monitored at 90°. In (c) the samples were in low salt buffer containing 5 mM $MgCl_2$. The emission monochromator bandwidth was varied between 1.5 and 3 nm. The room temperature samples were contained in 10 × 10 mm fluorescence cuvettes; and the 77 K samples in 2 mm inner diameter spectrosil tubes immersed in liquid N_2. Each sample was diluted until no further change in the shape of the spectrum was seen, when self-absorption could be discounted (the chlorophyll concentration was about 1 μg/ml).

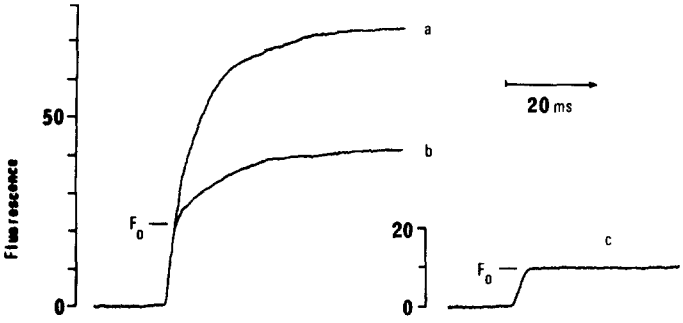

Fig. 3. Effect of $MgCl_2$ on chlorophyll fluorescence yield induction curves in dark-adapted chloroplasts from wild-type and *chlorina* f-2 mutant barley. The hypotonically broken chloroplasts were suspended in low salt buffer, pretreated with far-red light, then 5 mM KCl and 10 μM DCMU were added in the dark. Illumination was with broad-band blue-green light (Schott BG-38, 2 mm, maximum transmission 350—550 nm; Calflex C heat filter), intensity 10 W/m^2, through a photographic shutter and the emission was monitored at 90° with an EMI 9558B photomultiplier protected by a Balzar B-40 695 nm interference/Schott RG-695, 2 mm cut-off filter combination. The fluorescence signal was digitised and stored by a 1000 channel Datalab DL905 transient recorder and presented on an X-Y plotter. Curves (a) and (b) are for the wild-type with and without $MgCl_2$ addition (5 mM) respectively. Curve (c) is for the mutant both with and without $MgCl_2$. The chlorophyll concentration was 8 μg/ml for all samples. The fluorescence is expressed on the same arbitrary linear scale for all curves. All measurements were made at room temperature. In the case of curve (c) the rise to the F_m level is so slow that it is not reached during the time interval shown, in contrast to curves (a) and (b). No correction has been made for the different proportion of blue-green light absorbed by the mutant compared to the wild-type because taken over the whole absorption envelope the difference is relatively small.

plasts exhibit a much slower rise (curve c), and the maximum fluorescence level is lower than that of the wild-type at the same chlorophyll concentration and under the same illumination conditions (Table I). As can be seen from these curves (Fig. 3), the assessment of the F_0 level presents some difficulties due to

TABLE I

CHLOROPHYLL FLUORESCENCE YIELD OF WILD-TYPE AND *CHLORINA* f-2 MUTANT BARLEY UNDER VARIOUS EXPERIMENTAL CONDITIONS

The hypotonically-broken chloroplasts were suspended in a low salt buffer containing 5 mM KCl, and the fluorescence was measured as described in Fig. 3. When $MgCl_2$ was added it was present at a concentration of 5 mM. The chloroplasts were dark-adapted by pre-illumination with far-red light followed by a dark period of at least 1 min. When DCMU was present, it was added at a concentration of 10 μM to the dark-adapted samples. Chlorophyll concentration: 8 μg/ml. Fluorescence intensity is expressed in arbitrary units; temperature = 22°C. Note that the F_0 level of the wild type in the presence of DCMU is liable to overestimation at the relatively high actinic light intensity used.

Conditions	No Mg^{2+}		Plus Mg^{2+}	
	Wild-type	Mutant	Wild-type	Mutant
— DCMU				
F_0	20	11	26	10
F_m	46	21	96	19
F_m/F_0	2.3	1.9	3.7	1.9
+ DCMU				
F_0	27	11	33	10
F_m	51	18	100	18
F_m/F_0	1.9	1.6	3.0	1.8

the relatively slow opening time of the shutter (3 ms), however, the estimation of F_0 in the absence of DCMU is more precise since the fluorescence intensity rises more slowly. There is no noticeable effect of Mg^{2+} addition upon the induction curve for the mutant chloroplasts (Fig. 3, curve c); Table I quantifies the fluorescence yields observed at the F_0 and F_m levels in the presence and absence of Mg^{2+} and DCMU.

Figs. 4, 5 and 6 show the fluorescence decay curves for the wild-type and mutant barley chloroplasts under various experimental conditions, i.e. F_0 and F_m with and without Mg^{2+} added. Although the excitation intensities used were below those required for spurious quenching by excition annihilation, none of the fluorescence decay curves can be described by a single exponential decay law. Variation of excitation intensity between $2 \cdot 10^{13}$ and $2 \cdot 10^{14}$ photons/cm^2 produced no observable change in the form of the fluorescence decay kinetics. In the past, we have used an empirical law of the form $\exp(-At^{1/2})$ to fit these curves; the right hand parts of Figs. 4, 5 and 6 show the fluorescence intensity plotted as a function of $t^{1/2}$. Although this type of decay law was only used to provide a simple means of fitting the curves [9], it has been taken to indicate a specific mechanism for energy transfer (see ref. 11 for a review). However, the non-exponential decay law is probably more an indication of the heterogeneity of the system since fluorescence emission should be observed

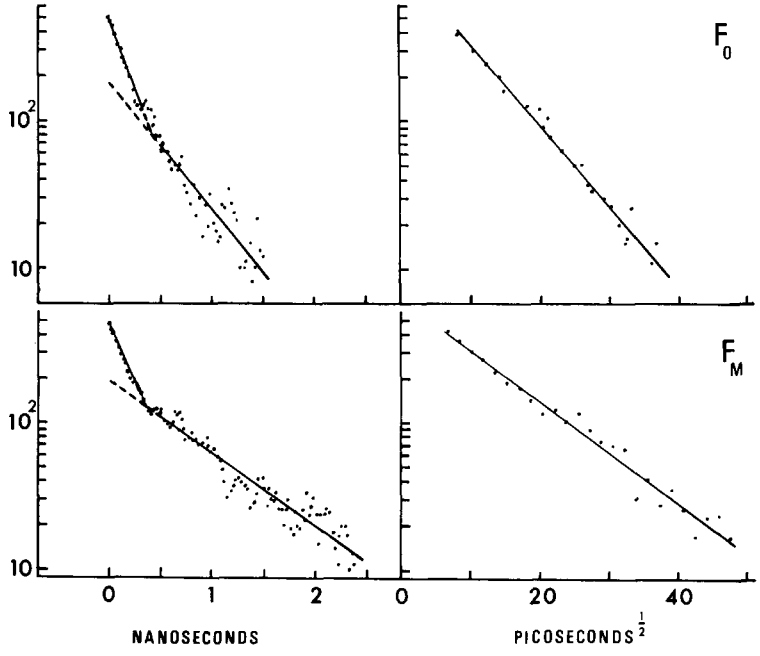

Fig. 4. Fluorescence decay kinetics of wild-type barley chloroplasts in low salt buffer in the absence of $MgCl_2$; for the dark-adapted state (F_0), and the maximum fluorescence level (F_m) seen on addition of DCMU and pre-illumination. Fluorescence is expressed on a logarithmic scale as the number of counts per channel. The measurements were made at a resolution of 55 ps on a timescale of 11.6 ps per channel. Other details are given in Table II. For clarity, only alternate data points have been plotted. The data represents the summation of 3 fluorescence traces recorded under the same conditions.

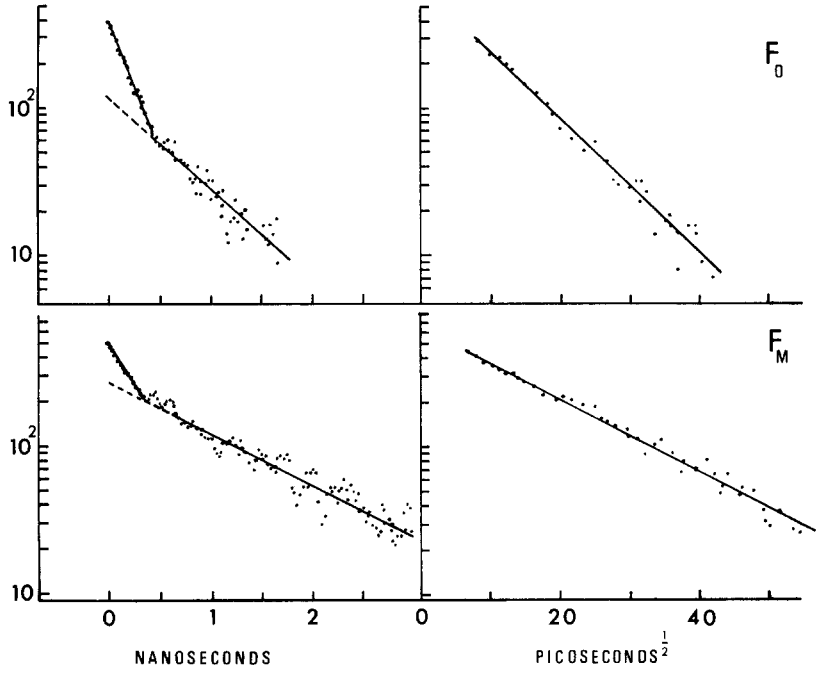

Fig. 5. Fluorescence decay kinetics of wild-type barley chloroplasts after addition of 5 mM $MgCl_2$. See Fig. 4 for further details.

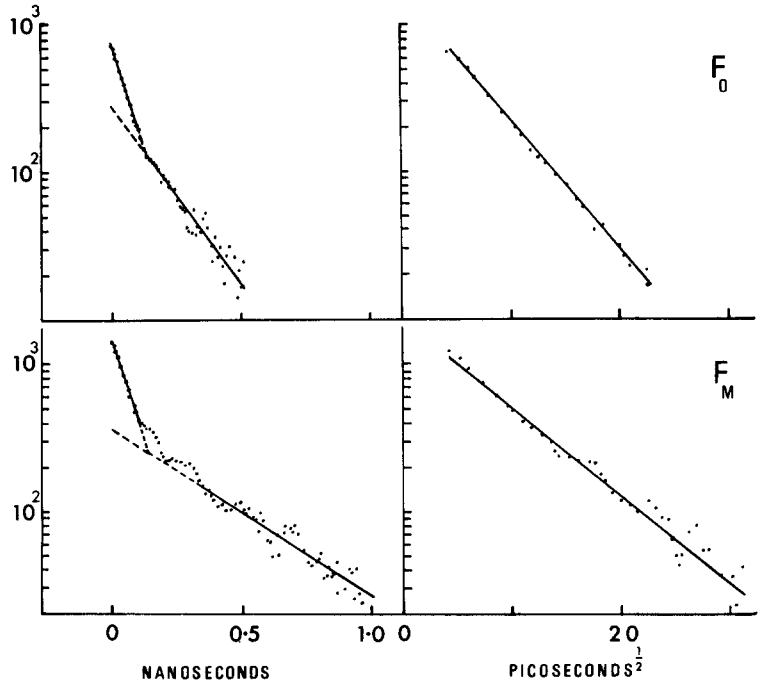

Fig. 6. Fluorescence decay kinetics of *chlorina* f-2 mutant barley chloroplasts in low salt buffer; addition of 5 mM $MgCl_2$ had no effect. The measurements were made at a resolution of 23 ps on a timescale of 4.62 ps per channel. Other details as for Fig. 4.

from Photosystem I as well as Photosystem II (see discussion). In this study, we have therefore returned to the more conventional fitting procedure using a dual exponential decay law; the general equation for the fluorescence intensity at time t, $I_F(t)$, is:

$$I_F(t) = I_F(0)[\alpha_1 \cdot \exp(-t/\tau_1) + \alpha_2 \cdot \exp(-t/\tau_2)]$$

where $I_F(0)$ is the fluorescence intensity at time $t = 0$, α_1 and α_2 are pre-exponential coefficients ($\alpha_1 + \alpha_2 = 1$) and τ_1 and τ_2 are the $1/e$ lifetimes of the two components. Table II summarises the values for these four variables along with the calculated fluorescence quantum yield (ϕ_{calc}) and the mean fluorescence lifetime (τ_M) for each of the experimental conditions used. It should be pointed out that the evaluation of the longer lived component, τ_2, is subject to a larger error than that of τ_1. A comparison between the fluorescence yield changes obtained from the kinetic and steady-state data is given in Table III. Since continuous illumination of the sample with the He/Ne laser causes excessive electron scatter within the streak camera, the beam was attenuated by a factor of 15 immediately before the time-resolved measurements were performed. Consequently, some of the reaction centres may have reopened, which may explain the relatively low value of F_m/F_0 calculated from the fluorescence decay curves particularly for the wild-type chloroplasts. The potential error in estimating F_0 from the fluorescence induction curves may also account for some of this discrepancy. Even so, our kinetic and steady-state yield data are both in reasonable agreement with those recently reported by Lieberman et al. [12].

The addition of DCMU and subsequent pre-illumination produces a large

TABLE II

FLUORESCENCE DECAY KINETICS OF WILD-TYPE AND *CHLORINA* f-2 MUTANT BARLEY

Fluoresence kinetics were measured on isolated chloroplasts with envelopes broken hypotonically and suspended in low salt buffer with the addition of 5 mM KCl (no Mg^{2+}) or 5 mM $MgCl_2$ (plus Mg^{2+}). Dark-adapted chloroplasts were used for F_0 measurements. At the F_m level chloroplasts were treated with 10 μM DCMU and pre-illuminated by the CW He/Ne laser, at full intensity for 30—60 s, and then attenuated 15-fold immediately before the time-resolved measurement. Temperature 20°C.

Conditions	Lifetimes (ps) *		Relative intensities		τ_M (ps) **	Φ_{calc} ***
	τ_1	τ_2	α_1	α_2		
Wild-type No Mg^{2+}						
F_0	157	598	0.77	0.23	258	0.0150
F_m	151	1062	0.72	0.28	402	0.0233
Wild-type plus Mg^{2+}						
F_0	146	617	0.66	0.34	306	0.0177
F_m	134	1290	0.52	0.48	690	0.0400
Chlorina f-2 mutant						
F_0	40	194	0.76	0.25	74	0.0043
F_m	60	424	0.80	0.20	135	0.0078

* Possible error in the lifetimes, ± 20%.

** The mean fluorescence lifetime (τ_M) is given by: $\tau_M = (\alpha_1 \cdot \tau_1 + \alpha_2 \cdot \tau_2)/(\alpha_1 + \alpha_2)$.

*** The fluorescence quantum yield (Φ_{calc}) is given by: $\Phi_{calc} = \Phi_0/\tau_0 I_F(0) \int_0^\infty I_F(t) \cdot dt$, where τ_0 = 5700 ps and $\Phi_0 = 0.33$ from chlorophyll *a* in vitro.

TABLE III

COMPARISON OF YIELD CHANGES OBSERVED IN THE STEADY-STATE, AND CALCULATED FROM KINETIC DATA, FOR WILD-TYPE AND *CHLORINA* f-2 BARLEY CHLOROPLASTS

The steady-state values are taken from Table I, for chloroplasts in the presence of DCMU. The kinetic values are taken from Table II (Φ_{calc}).

	Steady-state	Kinetics
F_m/F_0		
wild-type, no Mg^{2+}	1.9	1.6
wild-type, plus Mg^{2+}	3.0	2.3
mutant	1.7	1.8
$+Mg^{2+}/-Mg^{2+}$		
wild-type, F_0	1.2	1.2
wild-type, F_m	2.0	1.7

increase in the value of τ_2; whereas the addition of Mg^{2+} appears to increase the initial intensity of the second component, α_2 (see Table II and Figs. 4—6). The values of τ_2 for the wild-type chloroplasts are in good agreement with previous measurements on green photosynthetic systems. For example, values of $\tau_2(F_0 + Mg^{2+}) = 0.6$ ns, and $\tau_2(F_m + Mg^{2+}) = 1.3$ ns for the wild-type barley chloroplasts (Table II) compare favourably with values of 0.5 ns (F_0) and 1.6 ns (F_m) previously reported for pea chloroplasts [10], a spinach sub-chloroplast fraction [13] and *Chlorella* [14] under the same conditions.

In the case of the *chlorina* f-2 mutant (Fig. 6), the fluorescence decays more rapidly than for the wild-type chloroplasts under the same conditions, although the qualitative effect of DCMU is the same (Table II).

Discussion

The power of picosecond time-resolved measurements in the investigation of pigment organisation and excitation energy transfer within photosynthetic systems has been demonstrated by recent publications [5,6,10]. However, some investigators still question the use of laser pulse intensities in the range of 10^{13}—10^{14} photons/cm^2 and attribute the non-exponentiality of the fluorescence decay at short times to residual exciton annihilation (e.g., ref. 15). It is therefore important to show that the data presented in Table II and Figs. 4—6 are not subject to exciton annihilation induced by the intensity of the excitation pulse ($<2 \cdot 10^{14}$ photons/cm^2).

There are several lines of evidence which point to the absence of exciton annihilation effects. As mentioned above, a reduction of the excitation intensity to $2 \cdot 10^{13}$ photons/cm^2 produced no observable change in the form of the fluorescence decay kinetics (single pulse excitation); this intensity is well below the threshold for exciton annihilation effects [16]. Moreover, previous studies of the variation of fluorescence lifetime with excitation intensity indicate that an initial lifetime of the magnitude of τ_1 (approx. 150 ps) should only be observed at intensities considerably higher than 10^{15} photons/cm^2 [14,16]. Also, where measurements were carried out under identical conditions using *Chlorella pyrenoidosa*, a lifetime identical to that previously observed at lower

photon densitites was obtained [14]. Another indication of the absence of intensity effects is the agreement between the yield changes obtained from the kinetic and steady-state yield data (Table III); the yield changes calculated from the kinetic data are extremely sensitive to intensity-induced quenching processes [14]. Finally, the loss of the chlorophyll *a/b*-light-harvesting complex in the mutant should make the photosynthetic units less sensitive to the intensity of excitation: the fact that both τ_1 and τ_2 lifetimes are shorter in the mutant compared with the wild-type suggests that the general form of the fluorescence decay curves are indeed characteristic of the chloroplasts.

The steady-state fluorescence yield data listed in Table I show that the fluorescence yield of chlorophyll *a* is considerably less in the chlorophyll *b*-less mutant than in the wild-type barley chloroplasts; this difference is particularly pronounced at the F_m level with Mg^{2+} added. Thus, from a steady-state point of view, a considerable proportion of the room temperature fluorescence must be attributable to the chlorophyll *a/b*-light-harvesting complex in higher plants. Moreover, the sensitivity of the system to the Mg^{2+} concentration in the suspending medium is only observed when the light harvesting complex is present; a similar observation has already been reported for the chlorophyll *b*-less mutant, chlorophyll *b*-deficient mutants and for greening systems where the light harvesting complex had not yet formed [12,17]. Since the kinetic and steady-state yield changes are in reasonable agreement, the reduced fluorescence yield from the mutant can be interpreted in terms of the exciton lifetime within the pigment bed. Indeed, a comparison of the fluorescence lifetimes for the wild-type and the mutant barley (Table II) clearly shows the marked increase in lifetime produced by the presence of the light harvesting complex. This observation can be interpreted in one of two ways; either the exciton spends a finite time within the light-harvesting pigment bed before being irreversibly transferred to the chlorophyll *a* antenna of Photosystem II, or the exciton is reversibly transferred between the light-harvesting and Photosystem II pigment beds as proposed in the tripartite model of Butler [18]. In either case, the chlorophyll *a* of the light-harvesting complex retains the excitation energy long enough to emit some fluorescence. Since the chlorophyll *a* emissions from the light-harvesting and Photosystem II antenna complexes are inseparable at room temperature, the time-resolved fluorescence appears to come from a continuous chlorophyll *a* pigment bed.

From a simple point of view, there is no reason to suppose that the fluorescence decay curves should follow an exponential decay law since there are at least two emitting species in the thylakoid membrane; namely the chlorophyll *a* of the Photosystem I antenna, and of the light-harvesting/Photosystem II antenna combination. If a fraction of the Photosystem I chlorophylls, with a fluorescence lifetime of 100 ps [13], is physically separate from the light-harvesting/Photosystem II chlorophylls (e.g. in the stromal lamellae), then a dual exponential fluorescence decay curve would be expected, with a Photosystem I component (100 ps) and a light-harvesting/Photosystem II component (F_0, 500 ps; F_m, 1500 ps). The relative ratios of these two components and the lifetimes observed will be determined by factors such as the state of the Photosystem II reaction centres and the extent of spillover between the light-harvesting/Photosystem II antenna and the granal Photosystem I antenna. It

should be pointed out that, although Photosystem I has a low steady-state fluorescence yield, the initial intensity of this component in the time-resolved fluorescence emission will be proportional to the quanta absorbed by this system; this may exceed 30% of the total emission at time zero, if the value of $\alpha = 0.32$ given by the tripartite model [18] is correct.

Previous studies have clearly shown that the addition of Mg^{2+} not only increases the fluorescence yield but also the fluorescence lifetime [10,19,20]. A qualitative consideration of the coefficients α_1 and α_2 for the wild-type barley chloroplasts (Table II) indicates that a significant proportion of the long-lived component (α_2, τ_2) is converted to the short-lived component (α_1, τ_1) when Mg^{2+} is absent; this appears to be connected with a marginal increase in the value of τ_1 as might be expected for a kinetic redistribution of excitation energy. Both steady-state and kinetic fluorescence data for the F_0 levels suggest that the addition of Mg^{2+} under these conditions produces a small increase in the fluorescence yield (about 20%; see Tables I and II).

In the absence of a definite model for the photosynthetic unit, the interpretation of these fluorescence lifetimes must of necessity remain qualitative, although the results presented here should prove useful in the formulation of such a model at a later date. In general, the fluorescence lifetimes of the long-lived component (τ_2), both in the presence and absence of Mg^{2+}, are in agreement with the values reported for the light-harvesting /Photosystem II pigment combination of other photosynthetic species [10,13,14]. The markedly lower value of τ_2 in the mutant must result from the loss of the light-harvesting complex, and can therefore be ascribed to the average exciton lifetime within the Photosystem II chlorophyll *a* antenna system. At the F_m level, the fluorescence lifetime of the Photosystem II chlorophyll *a* might be expected to increase to a value of 4—5 ns, as found for an isolated chlorophyll-protein complex without reaction centres (see ref. 21). The absence of such a long lifetime in the barley mutant could either indicate that the closed Photosystem II reaction centre is still a relatively efficient quencher, or that energy can migrate from the Photosystem II antenna to the Photosystem I antenna in the absence of the light-harvesting complex. The short-lived component (τ_1) observed in the fluorescence from the mutant is subject to a potentially larger error, however, the approximate value of 50 ps is comparable to the 100 ps or less expected for the emission from Photosystem I [13]. Similarly, the relative initial intensities (α_1, α_2) are comparable to the relative absorbances expected for the antennae of Photosystems I and II in the absence of the light-harvesting complex [18].

The relative initial intensities (α_1, α_2) for the wild-type barley chloroplasts probably have less significance since the fluorescence decay curve is presumably composed of more than two exponential components; additional components could arise from the coupling between the light harvesting chlorophyll *a/b* and the antenna of Photosystem I even in the presence of Mg^{2+}, as suggested in the tripartite model of Butler [18]. The short-lived component (τ_1) must therefore be considered as a mixture of the emissions from the Photosystem I antenna (particularly from the stromal lamellae) and from those light-harvesting/Photosystem II pigment beds that are strongly coupled with Photosystem I in the granal lamellae. Consequently, the τ_2 components can be ascribed to those light-harvesting/Photosystem II pigment beds which are not

coupled with Photosystem I, either because of a large separation distance of for some reason unknown at present.

Conclusions

The data presented in this paper are perhaps the best indication yet reported that the fluorescence decay kinetics of photosynthetic systems are not governed by a single exponential decay law. Commensurate with our better understanding of the various deactivation pathways for excitation energy, we have replaced the purely empirical $\exp(-At^{1/2})$ decay law with a dual exponential decay law which appears to have more significance in this context. Eventually, further components will be required to describe each deactivation process in detail, and to denote the emission from all components of the thylakoid membrane. In terms of a possible model for pigment organisation, the majority of the data is consistent with the tripartite model of Butler [18], although the efficiency of spillover in the presence and absence of Mg^{2+} appears to be much higher than that predicted by steady-state measurements at 77 K.

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