Title

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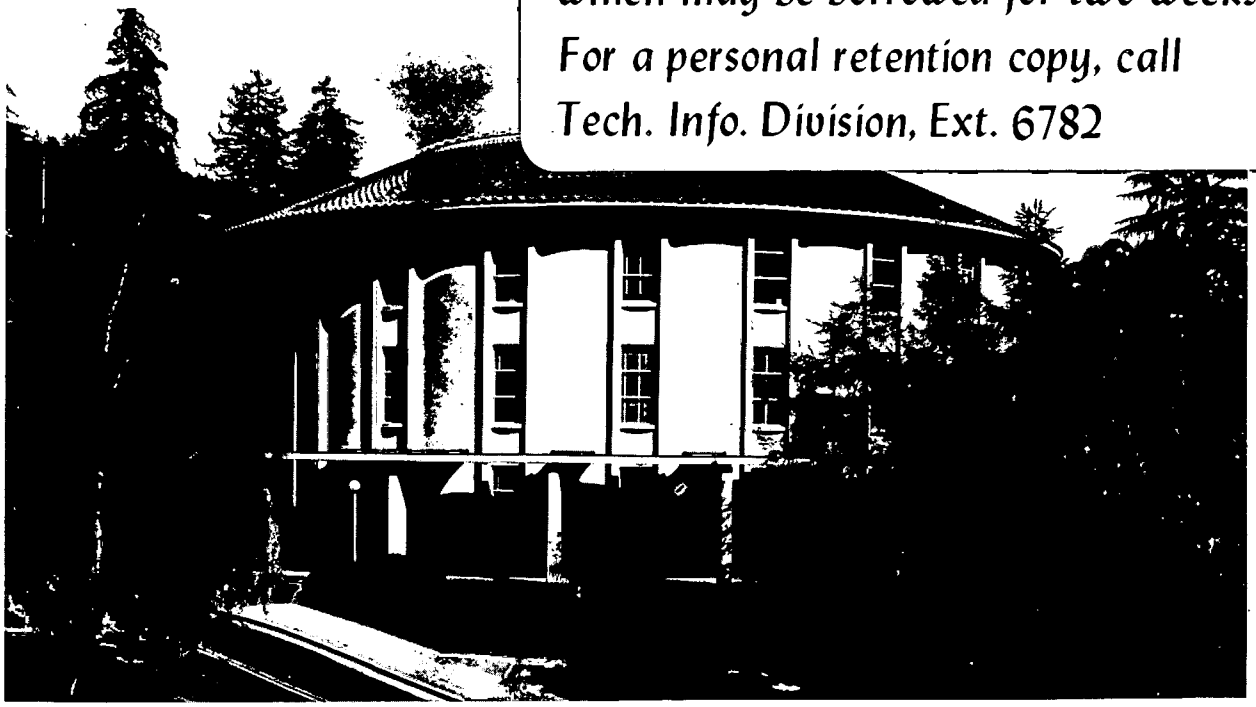

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DISCLAIMER

FLUORESCENCE LIFETIMES OF IN VIVO CHLOROPHYLL STUDIED IN CHLOROPLASTS AND ALGAE

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Fluorescence lifetimes of in vivo chlorophyll a in isolated chloroplasts and green algae have been investigated by single photon counting with picosecond time resolution. The fluorescence decay showed three exponential components in all species. Inactivation of the reaction centers II caused almost exclusively a 20-fold increase in the yield of the slowest component ($\tau \approx 2.2$ ns). We conclude that this variable part of the fluorescence is mainly controlled by a rate-limiting charge recombination in the reaction centers but that it indicates also energy transfer between photosynthetic units.

INTRODUCTION

In higher plants electronic excitation energy is transferred from photosynthetic units of ca. 400 chlorophylls to two reaction centers where it is converted to chemical energy by charge separation. In the open state of photosystem II the reaction centers are photoactive and the fluorescence yield is small, F_o. In the closed state no photochemistry is possible and the fluorescence yield is large, F_{max}. Time-resolved fluorescence measurements provide an approach to study the cooperation between photosynthetic units. Isolated units would show decay components with distinct lifetimes, one for units with open and one for those with closed reaction centers. Units in a matrix arrangement with energy transfer would show a continuous increase in the lifetime when the reaction centers become progressively closed (cf. ref. 1).

Another aspect is the origin of the fluorescence. A portion of the F_o fluorescence has been suggested to originate from chlorophylls which do not interact with photosystem II (2). In alternative models (3, 4) a homogeneous emission is presumed with F_o representing the residual decay of quanta which escaped the trapping in the reaction centers. But then the ratio of $(F_{max} - F_o)/F_{max} = 0.7$-$0.8$ (5) found in plants is too low compared to the maximum quantum yield in the reacting centers of ≈ 0.95 (6). Special deactivation paths (3, 4) have been proposed to account for this apparent anomaly. The goal of our measurements is to discriminate these possibilities from the time resolved fluorescence decay at high resolution and at low laser pulse energy which avoids singlet annihilation effects (7).

MATERIALS AND METHODS

Chloroplasts were isolated freshly from spinach leaves. The measuring solution (20-22°C) contained 10 mM HEPES-NaOH buffer, pH 7.5, 5 mM NaCl, 18 µg chlorophyll per ml and if indicated 5 mM $MgCl_2$. The reaction mixture was stirred in a 1x1 cm cuvette. Closed reaction centers were produced by illumination with flashes after addition of 12.5 µM diuron and 2 mM NH_2OH. The photon timing system employed is

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an improved version of that described (8). Pulses at 620 nm of $2\text{-}6 \cdot 10^6$ photons cm^{-2} and ~~fluorescence lifetimes of in vivo chlorophyll~~ <15 ps FWHM were obtained at a rate of 82 MHz from a R-6G dye laser synchronously pumped by a mode-locked Ar^+-laser (Spectra Physics 375 and 171, resp.). The fluorescence decay at 680 nm was deconvoluted from the response of the photomultiplier (RCA 31034A) by the least squares method.

RESULTS

Fig. 1 shows a measurement of the fluorescence decay $F_o(t)$ in spinach chloroplasts with open reaction centers. For the deconvolution of the fluorescence decay from the excitation profile E(t) we assumed a sum of exponential decay components. Fig. 1 shows in the middle and at the bottom the deviations of the best fit of a two- and a three-exponential decay, respectively, from the measured fluorescence decay. The deviations of the two-exponential fit near the maximum fluorescence intensity being typical for most of our measurements may have been missed

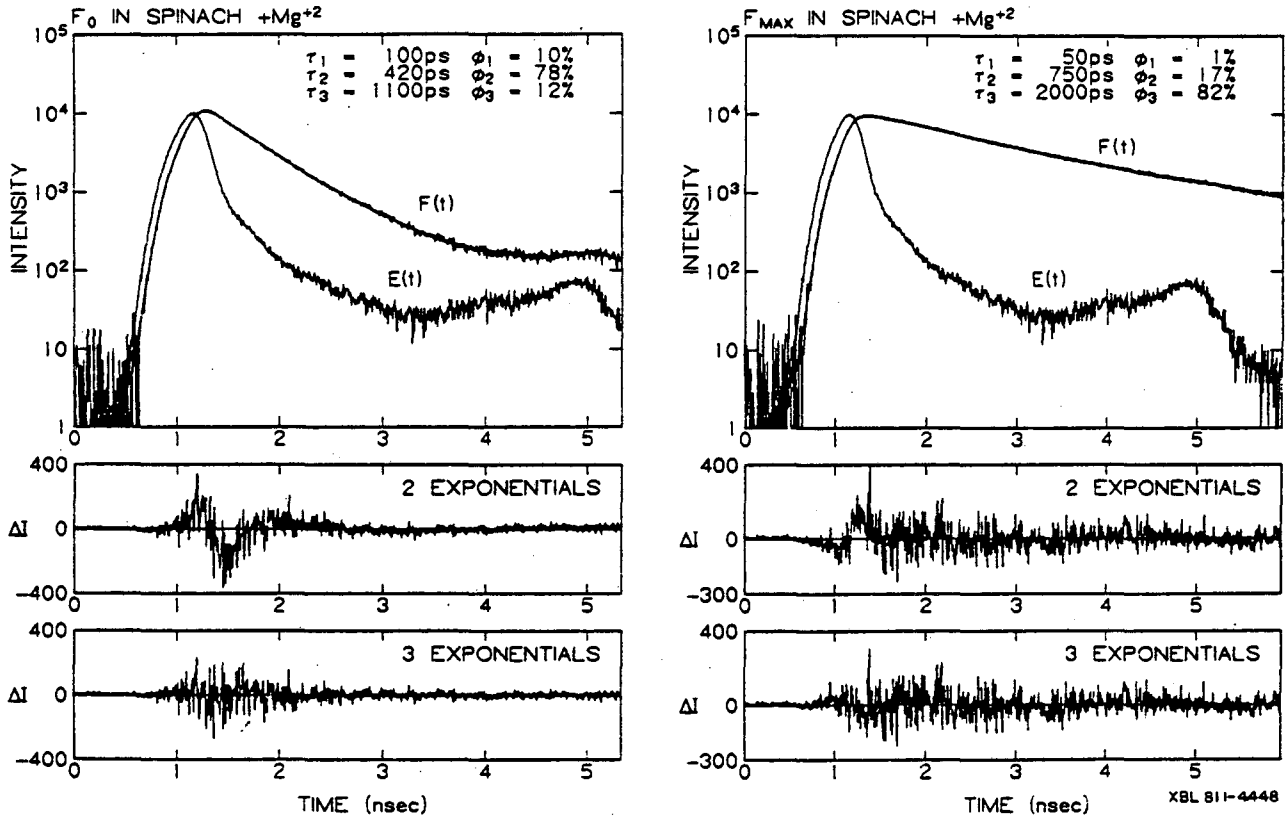

Fig. 1 Fluorescence decay of spinach chloroplasts at 680 nm at low excitation intensity (F_o level). The curve labelled E(t) is the excitation profile induced by the laser pulse. The curves labelled F(t) are the experimental fluorescence decay (noisy) and the calculated fit of a three-exponential decay (smooth), essentially superimposed. The lifetimes τ and the relative yields ϕ of the three kinetic components are indicated. Middle and bottom, deviations of the calculated best fit of a two- and a three-exponential decay from the experimental fluorescence decay, respectively, on a linear scale. The measuring solution contained 5 mM $MgCl_2$ and in addition 1.25 mM ferricyanide, 1.25 mM ferrocyanide and 10 µg gramicidin D. The distance between the channels was 8.2 ps.

Fig. 2 Fluorescence decay of spinach chloroplasts at 680 nm at the maximum fluorescence level F_{max}. Measuring solution with 5 mM $MgCl_2$ as in Materials and Methods. Other details as in Fig. 1

previously (9). The best fit of a three-exponential decay shows deviations only due to statistical noise. Fig. 2 shows the fluorescence decay $F_{max}(t)$ in spinach chloroplasts with closed photosystem II reaction centers.

The curves shown in Figs. 1 and 2 were measured in the presence of 5 mM $MgCl_2$. If divalent ions are absent large changes in the distribution of the light-harvesting chlorophyll a/b protein complexes have been observed. Therefore we have studied the fluorescence decay under these conditions. As in the presence of $MgCl_2$ only a three-exponential decay was sufficient to describe the fluorescence kinetics. The values of the lifetimes τ_i and the yields ϕ_i are included in the Table. In addition the data found with the green algae Chlamydomonas reinhardtii and Chlorella pyrenoidosa are shown for F_o and F_{max}. The lifetimes and yields of the three kinetic components are almost the same as those in chloroplasts except for a larger relative yield and a shorter lifetime of the slow component at open reaction centers.

DECAY COMPONENTS OF THE FLUORESCENCE KINETICS IN SPINACH CHLOROPLASTS AND ALGAE

species, conditions		τ_1 / ps	ϕ_1	τ_2 / ps	ϕ_2	τ_3 / ps	ϕ_3	ϕ_{tot}	τ_{mean} / ns
spinach chloroplasts, 5 mM NaCl	F_o	130	26	360	55	1500	19	100	0.55
	F_{max}	160	21	530	121	1700	116	258	1.04
spinach chloroplasts, 5 mM NaCl + 5 mM $MgCl_2$	F_o	110	15	420	114	1200	18	147	0.47
	F_{max}	50	6	750	100	2000	480	586	1.73
Chlamydomonas reinhardtii	F_o	70	8	390	44	750	48	100	0.54
	F_{max}	60	2.3	850	62	2300	310	374	2.05
Chlorella pyrenoidosa	F_o	60	2	390	30	840	68	100	0.69
	F_{max}	70	2.2	810	48	2100	250	300	1.88

The relative yields ϕ_i are normalized to the total yield ϕ_{tot} of the F_o fluorescence in the absence of $MgCl_2$. F_{max} in the algae was measured after 10 min incubation with 20 µM diuron and 10 mM NH_2OH as in chloroplasts. For further details see Figs. 1 and 2 and text.

DISCUSSION

The increase in the total fluorescence yield ϕ_{tot} when the reaction centers become closed is in agreement with the known ratio F_{max}/F_o = 3-5 (5, 10). For comparison with previous measurements we have calculated the averaged lifetimes $\tau_{mean} = (\sum_{i=1}^{3} \tau_i \phi_i)/\phi_{tot}$. The lifetimes found with phase fluorimetry by assumption of a single exponential decay (10) agree closely with our values of τ_{mean}. We confirm the proportional increase of τ_{mean} with ϕ_{tot} as measured with this technique. This apparent relation was believed to indicate an energy transfer between photosynthetic units (matrix model) and has been implicated in several models (3, 4). However, our results show as the dominant effect a dramatic increase in the yield of the slow component by a factor of 20-30 (in chloroplasts with $MgCl_2$) while the lifetime increases only from 1.1 to 2 ns. In a first approximation the fast component with a lifetime of ca. 100 ps and the middle component with 400-800 ps can be considered to be constant. Thus, the variable part of the fluorescence is apparently dominated by the slow component.

Our results are consistent with the model proposed by Klimov et al. (11), which describes the variable portion of light emission as luminescence after a rate-

limiting charge recombination between the primary electron donor and acceptor of photosystem II when the reaction centers are blocked by reduction of the secondary electron acceptor. The increase in the lifetime τ_3 indicates that energy transfer to other reaction centers is possible after the charge recombination. The fast and the middle component may represent fluorescence from chlorophyll antenna of the reaction centers. Their lifetimes reflect average energy transfer times from various parts of the antennae to the reaction center of photosystem II. At least some of the fast component may be fluorescence from photosystem I antennae as suggested by the lifetime of 110 ps found in photosystem I particles (12).

The effect of 5 mM $MgCl_2$ is to increase the yield of the slow component at closed reaction centers (F_{max}) by a factor of 2-3 while the lifetime is not changed significantly. In terms of the proposed mechanism this indicates a decrease in the rate of radiationless deactivation, possibly accompanied by an increased effective crossection of photosystem II antennae (5), and no change in the rate of the charge recombination.

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