A PICOSECOND PULSE TRAIN STUDY OF EXCITON DYNAMICS IN PHOTOSYNTHETIC MEMBRANES

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ABSTRACT The fluorescence decay time of spinach chloroplasts at 77°K was determined at 735 nm (corresponding to the photosystem I emission) using a train of 10-ps laser pulses spaced 10 ns apart. The fluorescence lifetime is constant at $\simeq$1.5 ns for up to the fourth pulse, but then decreases with increasing pulse number within the pulse train. This quenching is attributed to triplet excited states, and it is concluded that triplet excitons exhibit a time lag of about 50 ns in diffusing from light harvesting antenna pigments to photosystem I pigments. The diffusion coefficient of triplet excitons is at least 300–400 times slower than the diffusion coefficient of singlet excitons in chloroplast membranes.

INTRODUCTION

The availability of picosecond laser pulses has prompted a number of research groups to study fast energy transfer processes and fluorescence kinetics in photosynthetic membranes (1–8). At low temperatures, e.g. at 77°K, the fluorescence of chloroplasts extracted from spinach leaves, or the fluorescence exhibited by whole algal cells such as *Chlorella pyrenoidosa,* is characterized by two prominent emission maxima at 735 and 685 nm. These maxima are due to emission from chlorophyll *a* molecules associated with pigment system I (PS I) and pigment system II (PS II), respectively (9). Recently, Butler and co-workers (10,11) have utilized standard fluorescence techniques to study the energy distribution and energy transfer pathways between the light harvesting antenna pigments and PS I and PS II. We have studied both the fluorescence yields at low temperatures (4,5,12,13) and fluorescence decay time (1,4; see also references 7 and 14), using single picosecond laser pulses as well as picosecond laser pulse trains.

Using picosecond pulse trains, Breton and Geacintov (5,13) noticed that the quenching of the fluorescence was more pronounced in PS I than in PS II. Furthermore, the ratio of the fluorescence yield at 735 nm relative to that at 685 nm decreased as the number of picosecond pulses in a train was increased from 4 to about 300. On the

other hand, using a single picosecond laser pulse, the intensity of the fluorescence yield for both PS I and PS II decreases in a parallel manner with increasing pulse intensity (4,12). The increased quenching within PS I when trains of pulses are used was attributed to the preferential accumulation of long-lived quenchers within this pigment system. These quenchers were identified as triplet excitons and ions. Their concentration increases as a function of time within a pulse train because their lifetimes are long compared to the spacing of the picosecond pulses (5–10 ns), and the length of the train (typically $< 1\ \mu s$). Recent work, however, indicates that the predominant quenching effect is due to triplets.[1]

Other results (4,12,15) utilizing single picosecond pulse laser spectroscopy indicate that the PS I pigments derive their excitation energy mainly by exciton transfer from the light harvesting pigments rather than by the direct absorption of photons. The rise time of the PS I fluorescence is ≈ 140 ps (15), indicating that the singlet excitons diffuse within the light harvesting pigment matrix of the photosynthetic membranes before being captured by the PS I pigment system responsible for the 735-nm emission. Furthermore, the lifetime of the 685-nm fluorescence is strongly dependent on the intensity of the pulse, whereas the 735-nm emission is independent of intensity at least for incident intensities below 10^{15} photons cm^{-2} per pulse (4). These results indicate that singlet-singlet exciton annihilation takes place within the light harvesting system, that the actual optical cross section of the PS I pigment system responsible for the 735-nm fluorescence is relatively small at 530 nm, and that there is a time lag of $\cong 140$ ps associated with the arrival of excitons from the light harvesting to PS I pigments. It should be noted that in this work we refer to the collection of pigments which give rise to the 735-nm fluorescence as the PS I pigments. However, Butler (16) has proposed that the 735-nm emission is due to a chlorophyll form C-705 which derives its energy by exciton transfer from PS I antenna pigments.

In this paper we have addressed ourselves to two questions: (*a*) Is there a time lag for the arrival of triplet exciton quenchers within PS I analogous to the 140 ps time lag for singlet excitons? In organic crystals the diffusion coefficients for triplets are frequently two orders of magnitude smaller than those of singlet excitons (17). In chloroplasts we expect this difference to be even larger because the triplet excitation is transferred between different chlorophyll-protein complexes whose average distances, and thus transfer times, are probably larger than those between chlorophyll molecules within a given chlorophyll-protein complex (18). Inasmuch as triplet energy transfer is due to an exchange interaction, whereas the singlet exciton transfer is a longer range process, the difference between singlet and triplet diffusion coefficients may be much larger in chloroplasts than in organic crystals. With the spacing between successive picosecond laser pulses being 10 ns with the Nd:YAG laser utilized in this work, such a slow buildup of long-lived quenchers within PS I might be detectable with a pulse train. (*b*) A decrease in the fluorescence yield may be due to a static or a

[1] J. Breton and N. E. Geacintov. Submitted for publication.

dynamic quenching process (20). In the latter case, which is characteristic of exciton annihilation described by kinetic rate equations (21), the lifetime of the fluorescence decreases with increasing intensity of the laser pulses. An example of this situation is the intensity-dependent fluorescence decay time at 685 nm (1,4). In static quenching, the fluorescence decay time is independent of intensity—there are no quenching pathways within the particular subsystem whose fluorescence is still observable, whereas the fluorescence from the remaining subsystems is completely quenched. The 735-nm fluorescence decay at 77°K, excited in the single pulse mode, is insensitive to the pulse intensity, indicating that the exciton-exciton annihilations which produce the quantum yield decrease take place within the light harvesting pigment system rather than in the PS I pigment system. A determination of PS I fluorescence lifetimes under conditions of quenching by long-lived quenchers (5,6,13) with a pulse train can thus provide further information on the mechanism of this quenching process.

We have utilized a train of 10 530-nm · s harmonic pulses derived from a Nd:YAG laser operating at 1,060 nm to determine the singlet exciton fluorescence (735 nm) decay times for pulse numbers 4, 6, 8, and 10 within this pulse train. The decay times decrease with increasing pulse number, whereas the decay profiles of the fluorescence are exponential, indicating that simple dynamic quenching decay kinetics are applicable for singlet-triplet exciton quenching within the PS I pigments. The results are interpreted quantitatively by integrating numerically the appropriate coupled kinetic equations, and it is shown that there is a time lag in the buildup of triplet excitons within PS I.

METHODS

The experiments were performed at the Los Alamos Laboratory using spinach chloroplast suspensions at 77°K. The fluorescence emission was viewed through a 735-nm (± 7 nm) interference filter. The fluorescence was excited with picosecond pulses from a frequency doubled and mode-locked Nd:YAG (Nd^{+3}:yttrium aluminum garnet) laser and a streak camera-optical multichannel analyzer combination. The train consisted of ten main pulses, each 20 ps in duration, each separated by 10 ns, and of variable intensity as described in the text. The triggering of the streak camera was appropriately delayed in order to view the fluorescence decay after the pulses selected for study. Additional experimental details may be found elsewhere (1).

RESULTS

The intensity profile of a representative pulse sequence is shown in graph form in Fig. 1 A and in numerical form in Table I. The fluorescence decay times for pulses 2, 4, 6, 8, and 10 are plotted in Fig. 1 B. Using single pulse excitation, repeated determinations of the fluorescence decay times at 735 nm show that the lifetimes are 1.5±0.4 ns. The lifetime values obtained for pulses 2 and 4 thus indicate that there is little or no dynamic quenching of the PS I fluorescence for these early pulses. Indeed, Breton and Geacintov (5) have shown that the preferential quenching within PS I attributable to long-lived quenchers within PS I is not very pronounced for sequences of

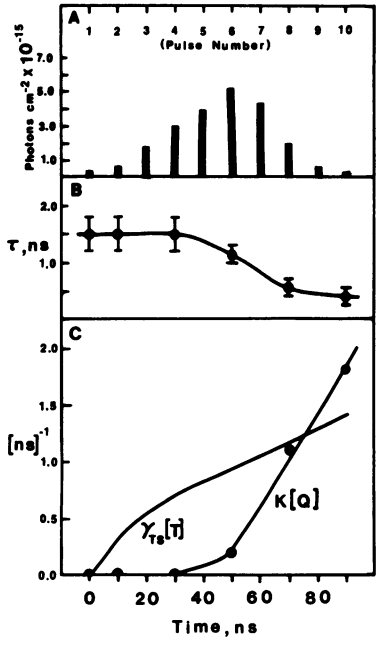

FIGURE 1

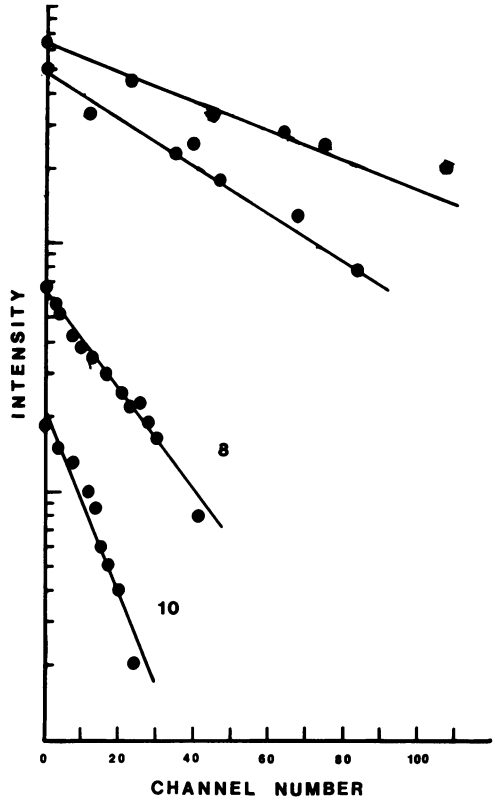

FIGURE 2

FIGURE 1 (A) Intensity profile of a typical pulse sequence. (B) Fluorescence (PS I) decay times measured at 77°K, 735-nm emission band. (C) $K[Q]$, quenching constant x quencher concentration within PS I calculated from experimental data using Eq. 1. $\gamma_{TS}[T]$—theoretical curve calculated from Eqs. 2–4. γ_{TS}— singlet-triplet exciton annihilation constant, $[T]$ is the triplet exciton density within the light harvesting pigments; the following parameters were used in this calculation: α = 1,400 cm^{-1}, experimental I_i values from Table I, $\beta_S = 1.3 \times 10^9$ s^{-1}, $\beta_T = 0.6 \times 10^5$ s^{-1}, $\gamma_{SS} = 5 \times 10^{-9}$ cm^3 s^{-1} (from ref. 12) $\gamma_{TS} = 7 \times 10^{-9}$ cm^3 s^{-1} $k_{is} = 1.3 \times 10^8$ s^{-1} (Geacintov et al. Submitted for publication).

FIGURE 2 Examples of semilogarithmic plots of the fluorescence decay. Data taken from digital readout of the optical multichannel analyzer display was utilized to process the streak camera traces. Scale is 20.8 ps per channel. Typical fluorescence decay curves for pulses 4, 6, 8, and 10 are shown. The lifetimes calculated from these particular traces are 1.5, 0.96, 0.47, and 0.25 ns, respectively.

four pulses or less. The fluorescence decay times decrease with increasing pulse number for pulses 6, 8, and 10. Fluorescence decay data taken from the digital readout of the optical multichannel analyzer are plotted on a semilogarithmic scale in Fig. 2. It is evident that within experimental error, the fluorescence decay is exponential. Under these conditions, the fluorescence decay times τ_i for pulses i = 6, 8, and 10, can be related to the decay time τ_o in the absence of quenching (for pulses 2 and 4, or single

TABLE I

INTENSITY OF INDIVIDUAL PULSES, FLUORESCENCE DECAY TIMES, $K[Q]$ (EQ. 1) AND CALCULATED VALUES $\gamma_{TS}[T]$ USING EQS. 2–4, $\gamma_{TS} = 7 \times 10^{-9}$ cm^3 s^{-1} AND $k_{is} = 1.3 \times 10^8$ s^{-1}.

Pulse number	Intensity, photons	Fluorescence lifetime	$K[Q]$	$\gamma_{TS}[T]$
	cm^{-2}	*ns*	$s^{-1} \times 10^{-9}$	$s^{-1} \times 10^{-9}$
1	0.23×10^{15}	1.5 ± 0.3	0	0.13
2	0.54×10^{15}	1.5 ± 0.3	0	0.32
3	1.8×10^{15}	—	—	0.55
4	2.9×10^{15}	1.5 ± 0.3	0	0.71
5	3.9×10^{15}	—	—	0.83
6	5.0×10^{15}	1.2 ± 0.2	0.17	0.90
7	4.3×10^{15}	—	—	1.0
8	1.8×10^{15}	0.57 ± 0.2	1.1	1.2
9	0.53×10^{15}	—	—	1.3
10	0.24×10^{15}	0.40 ± 0.15	1.8	1.4

The values given are averages of five or six determinations with their standard deviations.

pulse values of 1.5 ns) by the well-known Stern-Volmer equation (20):

$$\tau_i^{-1} - \tau_o^{-1} = K[Q]_i, \tag{1}$$

where $[Q]_i$ is the concentration of quenchers for pulse i, and K is the dynamic quenching constant.

DISCUSSION

The absorption coefficient of 530 nm can be estimated from the data of Schwartz (22) and the known absorption spectrum of chloroplasts. We estimate that the absorption coefficient α at 530 nm is about 1,200–1,400 cm^{-1}. With such an absorption coefficient, about 10% of the total chlorophyll molecules, on the average, are excited at an intensity of 5×10^{15} photons cm^{-2}/ps pulse (12), e.g. pulse 6. At these intensities, there is a severe decrease in the lifetime of the PS II fluorescence (1,4); even for pulse 1 (2.3×10^{14} photons cm^{-2} per pulse), the PS II fluorescence is already quenched (2). The fluorescence lifetime for PS I, on the other hand, begins to decrease significantly only with pulse 6. Using Eq. 1, we can calculate the product of the quenching constant K and the quencher concentration $[Q]$ in PS I. The results are plotted in Fig. 1 C. It is evident that the quencher concentration $[Q]$ in PS I begins to build up rapidly after pulse 6, whereas pulses 3–5 do not appear to give rise to any discernable quenching, at least immediately after the occurrence of these pulses. It, therefore, seems that the appearance of the quenchers Q within PS I has a time lag of about 50 ns.

To show that this time lag is real, we will now calculate, using the standard set of kinetic equations (1, 2, 4) below, the concentration of the quenchers in the light-harvesting antenna pigment system as a function of time. We assume that the quenchers $[Q]$ are triplet excitons (T), which are formed from singlet excitons (S) by inter-

system crossing with a rate constant k_{is} and eventually migrate to PS I. The singlet excitons can annihilate each other with a rate constant γ_{ss}, and the singlet excitons can also be annihilated by triplet excitons with a rate constant γ_{TS}. Rahman and Knox (23) estimate that $\gamma_{TS} = 6 \times 10^{-9}$ cm^{3}s^{-1} (23). The simple basic set of equations in this model then are

$$\frac{d[S]}{dt} = G(t) - \beta_S[S] - \gamma_{TS}[T][S] - \gamma_{ss}[S]^2 \tag{2}$$

and

$$\frac{d[T]}{dt} = k_{is}[S] - \beta_T[T]. \tag{3}$$

$\beta_S(s^{-1})$ denotes all unimolecular decay constants of singlets including photochemical pathways, where β_T denotes all unimolecular decay rates of triplets. The bimolecular triplet-triplet annihilation rates are neglected here. This approximation is justified on the time scales used because of the low probability of this process on the nanosecond time scale (this is shown for high triplet exciton densities in organic crystals in reference 24). $G(t)$ is the laser generating function

$$G(t) = \alpha \sum_{i=1}^{10} I_i \delta(t_i - t), \tag{4}$$

where $\alpha(cm^{-1})$ is the absorption coefficient and I_i the intensity (photons cm^{-2}) of the pulse incident on the sample, and the delta function takes into account the discontinuous nature of the excitation.

We have recently shown that the sets of Eqs. 2 and 3 can quantitatively describe the time dependence of the fluorescence quantum yield and triplet concentrations in spinach chloroplasts using an approximately square wave continuous (0.50 μs) laser pulse excitation.[2] These quantities were experimentally measured with a 50-ns resolution within the excitation pulse using a gated optical multichannel analyzer arrangement, whereas the $[S]$ and $[T]$ concentrations were calculated using Eqs. 2 and 3 utilizing the Hartree approximation. Excellent agreement between theory and experiment is obtained if the $\gamma_{ss}[S]^2$ term in Eq. 2 is neglected (justifiable when a microsecond pulse excitation is used because the lifetime of singlet excitons is <1 ns), and if values of $\gamma_{TS} = (7 \pm 2) \times 10^{-9}$ cm^3 s^{-1} are taken. These values of γ_{TS} are incidentally in excellent agreement with those calculated by Rahman and Knox (23).

Using the same approach, but retaining the $\gamma_{ss}[S]^2$ in Eq. 2 because of the picosecond nature of the excitation used in this work, we now calculate the triplet concentration for each of the pulses. Because the unimolecular triplet decay time $\beta_T^{-1} > 10^{-6}$ s(19), the triplets produced by the pulses accumulate from pulse to pulse during the train of pulses. In these calculations, we assumed that during the first pulse,

[2]Geacintov, N. E., J. Breton, and C. E. Swenberg. Submitted for publication.

$[T] = 0$ and thus the $\gamma_{TS}[T][S]$ is zero for this initial point. Then we calculate $[S]$ and, using 10^{-10}-s intervals, we substitute this value into the equations and calculate $[T]$ for subsequent 10^{-10}-s intervals; the total triplet concentration produced by pulse 1 is then added to the triplets produced by pulse 2, etc. Thus, by using stepwise numerical integration, using the values of $[T]$ obtained in the previous 10^{-10}-s interval, and treating these values of $[T]$ as constants in the succeeding interval, both $[S]$ and $[T]$ can be calculated for each of the 10 pulses. The values of $\gamma_{TS}[T]$ thus calculated, using $\gamma_{TS} = 7 \times 10^{-9}\text{cm}^3\text{s}^{-1}$, are plotted in Fig. 1 C.

Numerically, the magnitudes of the experimental quantities $K[Q]$ and $\gamma_{TS}[T]$ agree fairly well; however, the vertical scale for the $\gamma_{TS}[T]$ curve can be shifted up or down, depending on the exact value of γ_{TS} chosen. Thus, for example, using $\gamma_{TS} = 10^{-8}\text{cm}^3\text{s}^{-1}$, $\gamma_{TS}[T]$ can be made equal to $K[Q]$ for the last pulse (number 10). However, more important, there is little change in the shape of the theoretical curve $\gamma_{TS}[T]$ when γ_{TS} is changed. Furthermore, the vertical scales of the two curves in Fig. 1 C should not be compared in any case because the calculation refers to the concentration of $[T]$ in the light harvesting pigments, whereas $[Q]$ is identified here with the triplet exciton concentration within PS I. Using single picosecond laser fluorescence quantum yield studies, we have shown that the concentration of singlet excitons in PS I is proportional to the singlet exciton concentration in the light harvesting antenna pigment system (12); this determination was made by integrating the fluorescence yield produced by single picosecond pulses of different intensities and showing that the ratio of the yields at 685 and 735 nm was independent of the pulse intensity (12). The results shown in Fig. 1 indicate that the concentration of triplets in PS I does not follow the calculated concentration of triplets in the light harvesting pigments. It should be emphasized that this conclusion does not depend on the nature of the model embodied by Eqs. 2 and 3. It is qualitatively evident from the simple fact that pulses 3, 4, and 5, which are not significantly less intense than the subsequent pulses, do not produce any concentration of $[Q]$ within the PS I pigment system. The calculation, whose results are plotted in Fig. 1 C and are shown in Table I, merely confirms this qualitative conclusion.

We propose that the time dependence of the accumulation of the quenchers within PS I is due to the time it takes for triplet excitons to diffuse to the PS I pigment system from the light harvesting pigments where most of the photons are absorbed. We estimate this time lag to be of the order of 50 ns. This time is about $50/0.14 = 360$ times longer than the diffusion time of singlet excitons to PS I (15). Assuming that the average diffusion lengths for triplet and single excitons from light harvesting to PS I pigments are about the same, we can estimate the relative diffusion coefficients of singlet and triplet excitons D_S and D_T in chloroplasts at 77°K using the expression $D_S t_S = D_T t_T$, where t_S and t_T are 0.14 and 50 ns, respectively. The ratio of D_T/D_S thus obtained is $1/360$. This is a reasonable result, as discussed in the Introduction.

We finally make a comment on the exponentiality of the fluorescence decay which, as pointed out above, indicates the dynamic nature of this quenching. If PS I is a pigment system consisting of a small number of chlorophyll molecules (so that the radius

of a unit is comparable to a singlet-triplet interaction distance $\cong$40–50Å), and if a triplet exciton is present in a given PS I unit, one would expect any singlet exciton arriving at this unit to be quenched with nearly 100% efficiency. The quenching then would be static, because those PS I units (or pigments associated with the PS I fluorescence) without triplets would display the normal 1.5-ns fluorescence decay time, whereas those with triplets would exhibit little or no fluorescence. Because this is not the case, PS I must be large enough so that diffusion of excitons and dynamic quenching are both operative; as indicated in Eq. 1, the monomolecular decay rate is competitive in magnitude with the quenching rate. Thus, it is estimated that the radius of such a typical pigment unit is larger than about 40–50 Å and therefore has a cross-section of $\geq 75 \times 10^{-14}\text{cm}^2$. Using a value of $\cong 2.2 \times 10^{-14}\text{cm}^2$ for the area of a chlorophyll molecule, we estimate that there are at least 30–40 chlorophyll molecules associated with a PS I unit (or pigment system associated with the 735-nm fluorescence). On the other hand, we have shown that the optical cross-section of PS I is small relative to the cross-section of the light-harvesting pigments (12). Inasmuch as one photosynthetic unit comprising both PS I and PS II consists of $\cong$600 chlorophyll molecules (18), the value of at least 30–40 molecules in a PS I unit derived here is still consistent with the relatively small optical cross-section of the PS I pigments (4, 12).

CONCLUSIONS

Picosecond laser spectroscopy, using a combination of single pulse and pulse train excitation, can provide important information about triplet and singlet excition dynamics and exciton distributions in photosynthetic membranes. The mutual annihilation of excitons in chloroplasts provides a dynamic probe for the topology of the membranes. Because singlet and triplet excitions have widely different lifetimes and migration velocities, they can be utilized as intrinsic dynamic probes on widely different time scales, by selectively chosing the appropriate mode of laser excitation.

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REFERENCES

1. CAMPILLO, A. J., V. H. KOLLMAN, and S. L. SHAPIRO. 1976. Intensity dependence of the fluorescence lifetime *in vivo* chlorophyll excited by a picosecond light pulse. *Science (Wash. D.C.).* **193**:227–229.
2. CAMPILLO, A. J., S. L. SHAPIRO, V. H. KOLLMAN, K. R. WINN, and R. C. HYER. 1976. Picosecond exciton annihilation in photosynthetic systems. *Biophys. J.* **16**:93–97.
3. YU, W., P. O. HO, R. R. ALFANO, and M. SEIBERT. 1975. Fluorescent kinetics of chlorophyll in photosystems I and II enriched fractions of spinach. *Biochim. Biophys. Acta.* **387**:159–164.
4. GEACINTOV, N. E., J. BRETON, C. E. SWENBERG, A. J. CAMPILLO, R. C. HYER, and S. L. SHAPIRO. 1977. Picosecond and microsecond pulse laser studies of exciton quenching and exciton distribution in spinach chloroplasts at low temperatures. *Biochim. Biophys. Acta.* **461**:306–312.

5. BRETON, J., and N. E. GEACINTOV. 1977. Quenching of fluorescence of chlorophyll *in vivo* by long-lived excited states. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **69**:86–89.
6. PORTER, G., J. A. SYNOWIEC, and C. J. TREDWELL. 1977. Intensity effects on the fluorescence of *in vivo* chlorophyll. *Biochim. Biophys. Acta.* **459**:329–336.
7. SEARLE, G. F. W., J. BARBER, L. HARRIS, G. PORTER, and C. J. TREDWELL. 1977. Picosecond laser study of fluorescence lifetimes in spinach chloroplast photosystem I and photosystem II preparations. *Biochim. Biophys. Acta.* **459**:390–401.
8. PASCHENKO, V. Z., S. P. PROTASOV, A. B. RUBIN, K. N. TIMOFEEV, L. M. ZAMAZOVA, and L. B. RUBIN. 1975. Probing the kinetics of photosystem I and photosystem II fluorescence in pea chloroplasts on a picosecond pulse fluorometer. *Biochim. Biophys. Acta.* **408**:143–153.
9. GOVINDJEE, and L. YANG. 1966. Structure of the red fluorescence band in chloroplasts. *J. Gen. Physiol.* **49**:763–780.
10. BUTLER, W. L., and M. Kitajima. 1975. Energy transfer between photosystem II and photosystem I in chloroplasts. *Biochim. Biophys. Acta.* **396**:72–85.
11. SATOH, K., R. STRASSER, and W. L. BUTLER. 1976. A demonstration of energy transfer from photosystem II to photosystem I in chloroplasts. *Biochim. Biophys. Acta.* **440**:337–345.
12. GEACINTOV, N. E., J. BRETON, C. E. SWENBERG, and G. PAILLOTIN. 1977. A single pulse picosecond laser study of exciton dynamics in chloroplasts. *Photochem. Photobiol.* **26**:629–638.
13. GEACINTOV, N. E., and J. BRETON. 1977. Exciton annihilation in the two photosystems in chloroplasts at 100°K. *Biophys. J.* **17**:1–15.
14. HARRIS, L., G. PORTER, J. A. SYNOWIEC, C. J. TREDWELL, and J. BARBER. 1976. Fluorescence lifetimes of chlorella pyrenoidosa. *Biochem. Biophys. Acta.* **449**:329–339.
15. CAMPILLO, A. J., S. L. SHAPIRO, N. E. GEACINTOV, and C. E. SWENBERG. 1977. Single pulse picosecond determination of 735 nm fluorescence risetime in spinach chloroplasts. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **83**:316–320.
16. BUTLER, W. L. 1978. Energy distribution in the photochemical apparatus of photosynthesis. *Ann. Rev. Plant Physiol.* In press.
17. SWENBERG, C. E., and N. E. GEACINTOV. 1973. Exciton interactions in organic solids. *In* Organic Molecular Photophysics. J. B. Birks, editor. Wiley & Sons Ltd., Chichester, Sussex. 489–564.
18. SAUER, K. 1975. Primary events and the trapping of energy. *In* Bioenergetics of Photosynthesis. Govindjee, editor. Academic Press, Inc., New York. 115–181.
19. SHUVALOV, V. A. 1976. The study of the primary photo-processes in photosystem I of chloroplasts: recombination luminescence, chlorophyll triplet state and triplet-triplet annihilation. *Biochem. Biophys. Acta.* **430**:113–121.
20. BIRKS, J. B. 1970. Photophysics of Aromatic Molecules. Wiley & Sons Ltd., Chicester, Suxxes. Chap. 10.
21. SWENBERG, C. E., N. E. GEACINTOV, and M. POPE. 1976. Bimolecular quenching of excitons and fluorescence in the photosynthetic unit. *Biophys. J.* **16**:1447–1452.
22. SCHWARTZ, M. 1972. Quantum yield determination of photosynthetic reactions. *Adv. Enzym.* **24**:139–146.
23. RAHMAN, T. S., and R. S. KNOX. 1973. Theory of singlet-triplet exciton fusion. *Phys. Stat. Sol. (b).* **58**:715–720.
24. GEACINTOV, N. E., M. BINDER, C. E. SWENBERG, and M. POPE. 1975. Exciton dynamics in α-particle tracks in organic crystals: magnetic field study of the scintillation in tetracene. *Phys. Rev. B.* **12**:4113–4134.

DISCUSSION

CHAIRMAN: We begin with a question from an anonymous referee: The statement in your Results (page 349) that there is no difference between 685 and 735 nm fluorescence as a function of pulse intensity seems to contradict the earlier statement that 735 nm fluorescence is quenched more easily than the 685 nm fluorescence.