LIFETIME OF FLUORESCENCE FROM LIGHT-HARVESTING CHLOROPHYLL *a*/*b* PROTEINS

EXCITATION INTENSITY DEPENDENCE

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ABSTRACT The fluorescence from a purified, aggregate form of the light-harvesting chlorophyll *a*/*b* protein has a lifetime of 1.2 ± 0.5 ns at low excitation intensity, but the lifetime decreases significantly when the intensity of the 20-ps, 530-nm excitation pulse is increased above about 10^{16} photons/cm^2. A solubilized, monomeric form of the protein, on the other hand, has a fluorescence lifetime of 3.1 ± 0.3 ns independent of excitation intensity from 10^{14}–10^{18} photons/cm^2/pulse. We interpret the lifetime shortening in the aggregates and the lack of shortening in monomers in terms of exciton annihilation, facilitated in the aggregate by the larger population of interacting chlorophylls.

INTRODUCTION

Higher plants contain complex systems for the collection and utilization of light energy. One approach to the understanding of these systems is to separate and isolate functional components of these systems, understand the components separately, and then infer the operation of the whole. The first steps of the photosynthetic process, the absorption of light and initial transfer of this energy, take place in photosystem II in a component called the light-harvesting or antenna complex, composed minimally of protein, chlorophyll, and probably lipid. CPII (a protein containing three [or four] chlorophyll *a* molecules, three chlorophyll *b*, and a single polypeptide chain with a molecular weight 30,000) is believed to be a substituent of this light-harvesting complex. The oligomeric state (monomer, dimer, tetramer) of this protein appears to depend on the method of isolation (see Thornber et al., 1979). Related to CPII is a complex termed LHC, isolated by Burke et al. (1978).[1] This complex is an oligomeric protein containing about 13 molecules of chlorophyll per 23,000 mol wt protein, about twice the ratio in CPII, and readily aggregating into large structures (Thornber et al., 1979). There is spectral and electrophoretic evidence that CPII can be produced from LHC by addition of 0.1 to 1% sodium dodecyl sulfate (SDS) (Burke et al., 1978; Brown, 1979).

Measurement of the decay of fluorescence excited by a short laser pulse can be used to

[1]Authors have used the term light harvesting complex (LHC) to describe a variety of entities which have a chlorophyll *a*/chlorophyll *b* ratio of ~ 1. LHC in this paper refers to that complex prepared by the method of Burke et al. (1978).

study the structure of photosynthetic systems because the fluorescence lifetime of an excited chlorophyll depends upon its surroundings. In intact leaves or chloroplasts, for example, lifetimes of various types of chlorophyll range from < 100 ps to ~ 1 ns — much less than the 5–8 ns characteristic of free chlorophyll (Seibert, 1978; Searle et al., 1979; Brody and Rabinowitch, 1957). Decreased lifetimes are caused by the transfer of energy to other chromophores of low fluorescence yield. When the excitation intensity is raised sufficiently, another type of transfer, previously unobservable (though present), can appear. This is caused by "collision" of two excitations migrating in a pool of chromophores. The outcome of such a collision (termed exciton annihilation) may be one or no excitations and the fluorescence lifetime will be reduced relative to lifetimes observed at low excitation intensities. A related effect (on yield) has been observed in chloroplasts (Geacintov et al., 1977), and chloroplast fractions (Searle et al., 1977). In brief notes, Searle et al. (1978) and Searle and Tredwell (1979) mention lifetime shortening in a light-harvesting preparation similar in absorption spectrum and apparent molecular weight to the Thornber CPII preparation. One also expects the occurrence of exciton annihilation to depend upon the number of chromophores among which excitation can move. The most general treatment of this size effect is that of Paillotin et al. (1979), whose model contains two limiting cases. (*a*) The Swenberg limit, $\gamma << 2k$, applies to domains of many chromophores (γ is the singlet-singlet exciton annihilation rate in s^{-1} and k is the monomolecular decay rate in the absence of annihilation.) This γ is related to the bimolecular continuum rate γ_{ss}, units $cm^3\ s^{-1}$, in the three-dimensional case by the domain volume, V: $\gamma = \gamma_{ss}/V$. The fluorescence decay becomes nonexponential at high excitation intensity and the quantum yield decreases. (*b*) The Mauzerall limit (Mauzerall, 1976), $\gamma >> 2k$, applies primarily to domains of few chromophores. The high-excitation intensity fluorescence decays very rapidly near $t = 0$ and afterwards is exponential with time constant k^{-1}; the quantum yield also decreases with increasing intensity. It should also be kept in mind that the absolute intensity needed to create two excitons in a small domain of chromophores is larger than that in a large domain. With only six chromophores, one out of three must be excited to make annihilation possible; with 1,000 chromophores, only 1 in 500 need be excited.

A persistent problem encountered in the study of fluorescence lifetimes in the presence of exciton annihilation has been the poor signal-to-noise ratio encountered at excitation intensities low enough to exclude annihilation effects. (Geacintov et al. [1977] succeeded in accurately measuring the fluorescence yield of chloroplasts over a wide range of excitation intensities. A yield decrease upon excitation increase is not clear evidence of exciton annihilation, however, even in a system of identical chromophores unless multiple excitation of chromophores is ruled out. See Geacintov et al. [1979]; Breton and Geacintov [1980]). We have improved the signal-to-noise ratio with a signal averaging system made possible by a new low-jitter trigger in the streak camera detector.

In this paper, we present the intensity dependence of the fluorescence lifetimes of purified light-harvesting chlorophyll *a*/*b* proteins in a monomeric form (CPII) and in an aggregated form (LHC) in order to answer three questions:

(*a*) What is the fluorescence lifetime of the light-harvesting *a*/*b* protein, and does it depend upon the method of preparation?

(*b*) Is there exciton annihilation in the *a*/*b* protein monomer with its small number of chlorophylls?

(*c*) Will aggregates of the *a/b* protein, presumably with many more chlorophylls in the "pool," show enhanced annihilation?

Our results are a lifetime of ~ 3 ns for the monomer, with no intensity dependence in the range studied, and an intensity-dependent fluorescence decay for the aggregate prepared by a different method. This updates a previous report (Nordlund and Knox, 1980).

MATERIALS AND METHODS

We obtained spinach leaves from a local supermarket and harvested pea plants (*Pisum sativum* var "Early Alaska") from a greenhouse after ~ 2 wk growth. CPII was prepared from spinach and pea leaves by the SDS electrophoretic procedure of Kan and Thornber (1976) as modified by Van Metter (1977). We used 1.0- and 0.5-cm Diam gels in the electrophoretic procedure and prepared samples for optical study by slicing the CPII band from the gel and pressing it between two glass microscope slides separated by a 1.5-mm piece of Teflon coated with silicone high vacuum grease. LHC was prepared from pea leaves by sucrose gradient centrifugation (Burke et al., 1978). The LHC suspension was studied in a 1-mm path length glass cuvette. We kept the samples dark and on ice until placement of the cuvettes on the slit of the streak camera or photodiode detection system. The LHC suspensions were shaken every few minutes to prevent settling of the particles. Optical densities of the samples were 0.2–1.2 at 672 nm (678 nm for LHC) and 0.03–0.17 at 530 nm. Fig. 1 shows the absorption spectra of the two preparations.

We excited fluorescence with single, 15–20-ps, 530-nm laser pulses from a frequency-doubled Nd:YAG (neodymium^{3+}: Yttrium Aluminum Garnet) laser, repetition rate 0.5 Hz, and detected at 180° with a low-jitter (<2 ps) streak camera-optical multichannel analyzer system described elsewhere (Mourou and Knox, 1979 and 1980; Stavola et al., 1980). We mounted samples at the focal point of a 6-cm focal length cylindrical lens. The line of excited fluorescence formed, in effect, the slit of the streak camera. Fluorescence on a longer time scale was detected at 180° with an EG&G FND-100 photodiode (EG&G Inc., Electro-Optics Div., Salem, Mass.) and Tektronix 7834 oscilloscope (Tektronix Inc., Beaverton, Ore.), risetime 1 ns. The excitation beam size was determined by a spherical 10-cm focal length lens set to focus or unfocus the beam. Glass neutral density filters in front of the lenses determined the photon density incident on the sample and Schott BG18 filters (Schott Optical Glass, Inc., Duryea, Pa.) blocked 1060-nm laser light. Fluorescence was filtered through Kodak Wratten gel neutral density (Eastman Kodak Co., Rochester, N.Y.) and Schott glass longpass filters placed behind the sample.

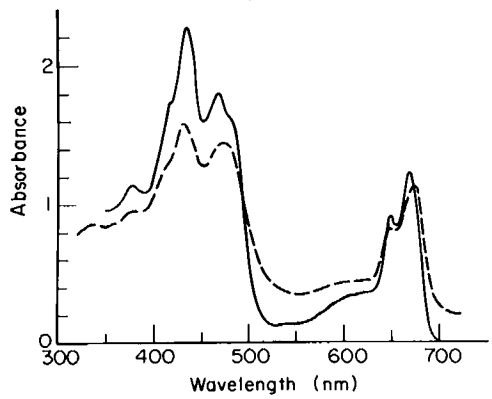

FIGURE 1 Absorption spectra of CPII (solid line) and LHC (dashed line) samples used in laser experiments; optical path lengths 1.5 and 1.0 mm, respectively. Scattering is evident in the LHC spectrum.

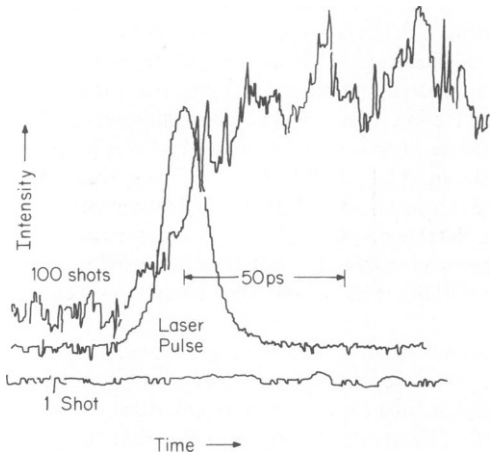

FIGURE 2

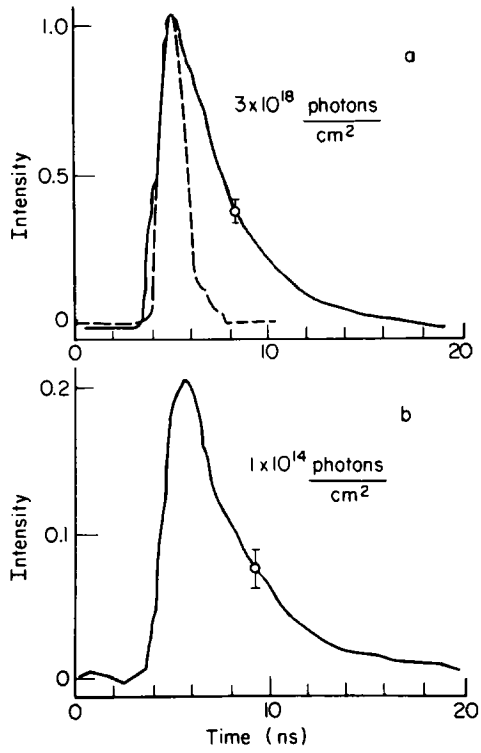

FIGURE 3

FIGURE 2 Signal-averaged fluorescence rise (arbitrary units) of LHC excited by a 50-nJ laser pulse. Bottom curve, 1 shot; top curve, sum of 100 shots at same amplification; middle curve, sum of 10 attenuated laser pulses. The low jitter of the detection system allows direct comparison of the time axes to < 2 ps without sliding and matching.

FIGURE 3 Fluorescence of CPII detected with a photodiode. (*a*) CPII fluorescence excited with 3×10^{18} photons/cm^2 (solid line); response of detector to an attenuated laser pulse (dashed line). (*b*) CPII fluorescence at 1×10^{14} photons/cm^2. The intensity scale in (*a*) is arbitrary, but that in (*b*) is related to that in (*a*) as shown. Fluorescence was filtered through a Schott RG630 filter. Curves were digitized and replotted from oscilloscope trace photos. The error bar is appropriate along the entire trace.

The low jitter of the streak camera trigger allowed us to perform simple addition of many single shots in the multichannel analyzer. Fig. 2 compares a single low-intensity shot with a summation of 100 such shots obtained from excitation of LHC aggregates.

RESULTS

CPII

The oscilloscope traces (Fig. 3) of fluorescence decay from spinach CPII detected with a photodiode show a lifetime of 3.1 ± 0.3 ns with no intensity dependence in the region 10^{14}–3×10^{18} photons/cm^2, corresponding to ~ 10^{-2} to (potentially) 10^2 photons absorbed/chlorophyll. The decay is exponential within the noise, though there may be a small fast component masked by the photodiode–scope response time, as shown by the streak camera traces in Fig. 4. The lifetime is unaffected when the cutoff wavelength of the longpass filter behind the

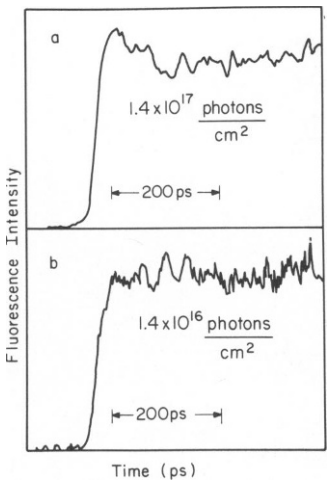

FIGURE 4

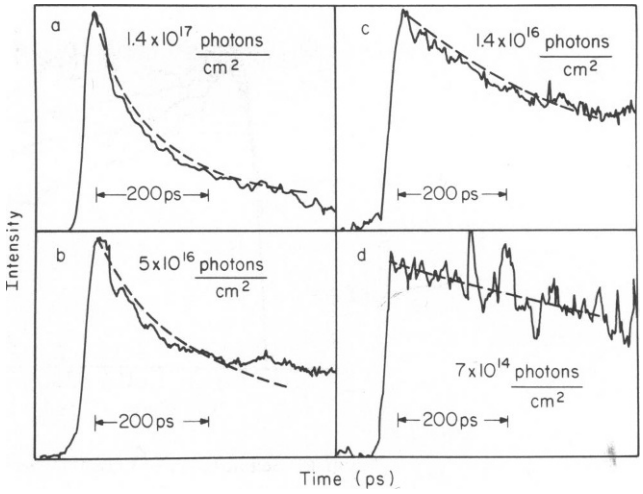

FIGURE 5

FIGURE 4 Fluorescence of pea CPII detected with the streak camera system. (*a*) Excitation intensity 1.4 $\times$ 10^{17} photons/cm^2; sum of five shots. (*b*) 1.4 $\times$ 10^{16} photons/cm^2; sum of 10 shots. The slight rise toward the end of each trace is caused by nonuniform response across the screen. Chlorophyll concentration about one-third that in Fig. 1.

FIGURE 5 Fluorescence decay (arbitrary units) of LHC as a function of excitation intensity. (*a*) Excitation intensity 1.4 $\times$ 10^{17} photons/cm^2; sum of 5 shots. (*b*) 5 $\times$ 10^{16} photons/cm^2; sum of 20 shots. (*c*) 1.4 $\times$ 10^{16} photons/cm^2; sum of 60 shots. (*d*) 7 $\times$ 10^{14} photons/cm^2; sum of 40 shots. Backgrounds were normally subtracted for each curve by recording without a laser pulse. Background for curve (*a*) consisted, instead, of 5 laser pulses fired into a part of the cuvette not containing LHC. The dashed curves are fits to the solution of Eq. 1, $N(t) = [(1/n(0) + \gamma_{ss}/2k)\exp(kt) + \gamma_{ss}/2k]^{-1}$, with $k = 8 \times 10^8$ s^{-1}, $\gamma_{ss} = 3 \times 10^{-9}$ cm^3 s^{-1}. The chlorophyll concentration within an aggregate has been taken equal to 1.5 $\times$ 10^{19} cm^{-3} and the exciton population at $t = 0$ has been calculated from the intensity and absorption and scattering cross sections. For *a*, *b*, *c*, and *d*, $n(0) = 7 \times 10^{18}$ cm^{-3}, 3×10^{18} cm^{-3}, 10^{18} cm^{-3}, and 5×10^{16} cm^{-3}, respectively. The amplitudes of all curves are normalized.

sample is varied from 570 to 630 to 645 to 695 nm and when the sample concentration is reduced by a factor 4. When the CPII was denatured[2] by heating to 50°C for 10 min the fluorescence intensity was reduced, but the lifetime remained at ~ 3 ns. The other two chlorophyll-containing bands produced on the electrophoresis gel, CPI, containing a photochemically inactive form of the reaction center from Photosystem I as well as some antenna chlorophylls, and free (presumably SDS-solubilized) chlorophyll have lifetimes of 4.5 ± 1.0 ns and show no intensity dependence. Searle et al. (1978) reported the same lifetime for a similar CPI preparation.

LHC

The fluorescence decay from the LHC aggregates is strongly dependent upon intensity. Rapidly decaying components develop at high intensity on top of the slow component (1.2 ± 0.5 ns) found at low intensity (Fig. 5). Fig. 6, a semilog plot of the decays, shows the nonexponential character at high intensity. The highest intensity curve can be fit approxi-

[2]A denaturation of CPII has been inferred by Van Metter (1977) from loss of fluorescence depolarization and by Shepanski and Knox (1981) from loss of exciton contributions to circular dichroism.

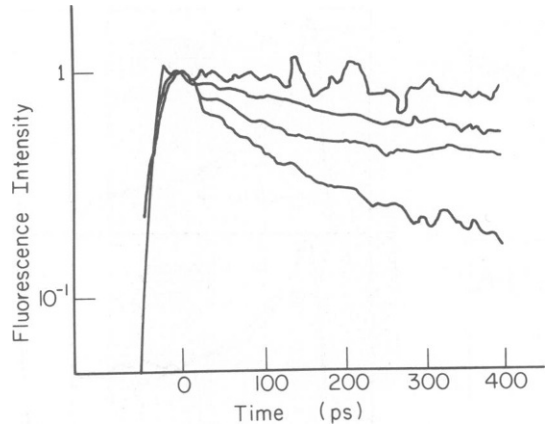

FIGURE 6 Semilog plots of LHC fluorescence from Fig. 5.

mately by a sum of two exponentials, 50% of the total with a decay time of 40 ps and 50% with 300 ps. The high intensity curves are unchanged (except for reduced signal-to-noise) when a Wratten neutral density 1.0 filter is placed behind the sample to cut the fluorescence entering the streak camera by a factor of 10. There is also no significant contribution to these signals from stray excitation light or filter fluorescence, as shown in Fig. 5*a*, where backgrounds were accumulated by measurements with a buffer-filled cell. The same degree of lifetime shortening was also observed when the low wavelength cutoff of fluorescence was raised from 570 to 695 nm and after the sample was left undisturbed in the refrigerator for 16 h, allowing most of the (larger) particles to settle to the bottom of the cell.

DISCUSSION

We have measured the time and excitation intensity dependence of the flourescence decay from two purified forms of the light-harvesting chlorophyll *a*/*b* protein: an aggregate and a solubilized, monomeric form. The monomer's decay is approximately exponential, with lifetime 3.1 ± 0.3 ns independent of the intensity of the 20 ps, 530-nm excitation pulse from 10^{14}–10^{18} photons/cm^2. At low intensity the aggregate lifetime is 1.2 ± 0.5 ns. These lifetimes are shorter than the free chlorophyll lifetime of 5–8 ns but rather longer than the major low-intensity in vivo decay component of 400–600 ps (Campillo et al., 1976; Beddard et al., 1979), reflecting the partial severing of dissipative pathways from the chlorophylls by physical isolation and purification of the chlorophyll protein. At excitation intensities above ~ 10^{16} photons/cm^2, the aggregate's decay becomes faster, with a 40-ps decay component at 10^{17} photons/cm^2.

We interpret the lifetime shortening in the aggregates and the lack of shortening in monomers in terms of exciton annihilation, facilitated in the aggregate case by an increased population of interacting chlorophylls and possibly by the greater number of chlorophylls per gram protein. Annihilation may also be occurring in the monomer if, because of close proximity of the chlorphylls, the process is fast compared with the laser pulsewidth.[3] The

[3]G. Paillotin, private communication.

amplitude of this fast process would be reduced by convolution with the 20-ps laser pulse. Fig. 4*a* shows some evidence of a small fast component present in the fluorescence decay excited with a high intensity. Use of a shorter excitation pulse or measurement of yield vs. intensity should resolve this question of singlet-singlet annihilation in the monomer. Though intensities of 5×10^{16}–5×10^{17} photons/cm^2 shorten the lifetime of chlorophyll *a* in pyridine solutions when excited at 337 nm by stimulating emission (Hindman et al., 1978), we believe this mechanism to be inoperative in LHC emission because of the lack of shortening in CPII. One expects the monomer to have a lower threshold for stimulated emission because of decreased scattering and decreased coupling to nearby chlorophyll proteins. Neglecting the particulate nature of the LHC, we also estimate gain thresholds (see for example Snavely, 1977; Dienes, 1975) that preclude lasing or significant single-pass amplified emission in our system. For these estimates we have included the absorption of the first excited singlet of chlorophyll *a* in pyridine, which Shepanski and Anderson (1981) measure to be 2.5 and 1.0 times that of ground state at 530 and 681 nm, respectively. This cross section is preferred to that of Baugher et al. (1979), because Shepanski and Anderson used picosecond excitation, eliminating deconvolution of the laser pulse and triplet populations. The threshold arguments will need reevaluation when it is determined which species (perhaps carotenoids or chlorophyll *b*; [Thornber et al., 1979]) absorbs most of the excitation light at 530 nm and how the energy is transferred (if necessary) to chlorophyll *a*. The kinetics will be further clarified when we monitor the angular dependence and spectral width of the emission and when we use excitation provided by a recently constructed stimulated Raman scattering system emitting pulses near 650 nm. For further discussion of stimulated emission effects in chlorophyll systems we refer the reader to Geacintov et al. (1979). When measuring fluorescence kinetics, possible artifacts caused by excited state absorption must also be checked. The effect of this absorption is indirect, in that the initial population of the first excited state may be altered at high intensity by excitation into and relaxation from higher states. The decay of the first excited singlet would apparently have a different rate parameter γ_{ss} in Eq. 1 below. However, computer simulations show that the change of the first excited singlet population that results from excited-state absorption is at most a few percent after the end of the excitation pulse and can thus be ignored (Shepanski and Nordlund, private communication). We note also that Breton and Geacintov (1980) report a reduction in the yield of CPII fluorescence as the intensity of a 1-μs, 637-nm dye laser pulse is raised. Excited triplets annihilate the singlet excitons in this case because of the long laser pulse.

A bimolecular annihilation rate constant can be estimated for LHC in the continuum limit if one integrates Swenberg's expression (see Swenberg et al., 1976, and Geacintov et al., 1977),

$$dn/dt = -kn - \gamma_{ss} n^2. \quad (1)$$

Estimating the chlorophyll molecule concentration within an LHC aggregate to be 10^{19}–10^{20} cm^{-3}, fits to the curves in Fig. 5 can be obtained with $k = 8 \times 10^8$ s^{-1}, $\gamma_{ss} = 0.5$–5×10^{-9} cm^3 s^{-1}. This compares to a value of $\gamma_{ss} = 5$–15×10^{-9} cm^3 s^{-1} obtained by Geacintov et al. (1977) for annihilation in chloroplasts. If we further follow the Paillotin et al. (1979) theory of annihilation we conclude from the shapes of the LHC decay curves that $\gamma = \gamma_{ss}/V << 2k$, where again V is the domain volume and k is the monomolecular decay rate in the absence of

annihilation. Putting in our values for γ_{ss} we then find that the number of chlorophylls in a domain volume is much > 30, consistent with the continuum approximation of Eq. 1. The associated domain volume, for a local chlorophyll concentration of 10^{19} cm^{-3} or 10^{20} cm^{-3}, must be much > $(16\ nm)^3$ or $(7\ nm)^3$, respectively. These volumes could contain, for example, many protein molecules of molecular weight 30,000, so that the domain in LHC encompasses many polypeptides. We cannot follow the same reasoning for the CPII data because we cannot get an estimate for γ_{ss}. The data can be interpreted in at least two ways. The first is to conclude that the Mauzerall limit $\gamma >> 2k$, discussed in the Introduction, applies and that fast annihilation takes place during the excitation pulse. That this limit applies to domains of few chromophores is consistent with the monomeric nature of the CPII preparation, in which each monomer contains six chlorophylls. An alternative explanation is that two excitons cannot exist in such a small domain. We believe that the three chlorophyll *b* in CPII are exciton coupled so that any excitation is delocalized among the chlorophyll molecules (Van Metter, 1977). In this case a second photon absorbed by the *b* trimer would simply excite the system into a higher trimer state. One may not be able to speak in terms of the simple picture of annihilation in this case because of the delocalization. The matter of annihilation in small systems needs further exploration. We plan to distinguish between the Mauzerall limit of annihilation, exclusion of multiple excitons, and other high intensity excitation effects in small systems by measuring to shorter times with a shorter (0.5 ps) excitation pulse, by simultaneously monitoring the fluorescence yield, and by exciting at advantageous wavelengths.

Quantum yields ϕ of fluorescence can be estimated from the low intensity lifetimes, $\phi = \tau/\tau_0$, assuming a value of 15 ns for τ_0 (Brody and Rabinowitch, 1957). This gives $\phi = 0.21 \pm 0.02$ for CPII and $\phi = 0.08 \pm 0.03$ for LHC. The yield reported for CPII by Knox and Van Metter (1979) was 0.1.

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REFERENCES

Baugher, J., J. C. Hindman, and J. J. Katz. 1979. Determination of the triplet and excited singlet absorption spectra of chlorophyll *a* with a tunable dye laser. *Chem. Phys. Lett.* 63:159–162.

Beddard, G.S., G. R. Fleming, G. Porter, G. F. W. Searle, and J. A. Synowiec. 1979. The fluorescence decay kinetics of *in vivo* chlorophyll measured using low intensity excitation. *Biochem. Biophys. Acta.* 545:165–174.

Breton, J., and N. E. Geacintov. 1980. Picosecond fluorescence kinetics and fast energy transfer processes in photosynthetic membranes. *Biochim. Biophys. Acta.* 594:1–32.

Brody, S. S., and E. Rabinowitch. 1957. Excitation lifetime of photosynthetic pigments *in vitro* and *in vivo*. *Science (Wash., D.C.).* 125:555.

Brown, J. S. 1979. Spectral studies of triton-solubilized chlorophyll proteins from *Euglena* and antenna chlorophyll from spinach. *Carnegie Instit. Wash. Year Book*. 78:189–194.

Burke, J. J., C. L. Ditto, and C. J. Arntzen. 1978. Involvement of the light-harvesting complex in cation regulation of excitation energy distribution in chloroplasts. *Arch. Biochem. Biophys.* 187:252–263.

Campillo, A. J., V. H. Kollman, and S. L. Shapiro. 1976. Intensity dependence of the fluorescence lifetime of *in vivo* chlorophyll excited by a picosecond light pulse. *Science (Wash., D.C.)*. 193:227–229.

Dienes, A. 1975. Dye lasers. *In* Laser Applications to Optics and Spectroscopy. S. Jacobs, M. S. Sargent, III, J. F. Scott, and M. O. Scully, editors. Addison-Wesley Publishing Co., Reading, Mass. 53–121.

Geacintov, N. E., J. Breton, C. E. Swenberg, and G. Paillotin. 1977. A single pulse picosecond laser study of excitation dynamics in chloroplasts. *Photochem. Photobiol.* 26:629–638.

Geacintov, N. E., D. Husiak, T. Kolubaev, J. Breton, A. J. Campillo, S. L. Shapiro, K. R. Winn, and P. K. Woodbridge. 1979. Exciton annihilation versus excited state absorption and stimulated emission effects in laser studies of fluorescence quenching of chlorophyll *in vitro* and *in vivo*. *Chem. Phys. Lett.* 66:154–158.

Hindman, J. C., R. Kugel, A. Svirmickas, and J. J. Katz. 1978. Stimulated fluorescence and fluorescence quenching in chlorophyll *a* and bacteriochlorophyll *a*. *Chem. Phys. Lett.* 53:197–200.

Kan, K. S., and J. P. Thornber. 1976. The light harvesting chlorophyll *a/b* protein complex of *Chlamydomonas reinhardii*. *Plant Physiol.* 57:47–52.

Knox, R. S., and R. L. Van Metter. 1979. Fluorescence of light-harvesting chlorophyll *a/b*-protein complexes: implications for the photosynthetic unit. *In* Chlorophyll Organization and Energy Transfer in Photosysthesis. *Ciba Found. Symp. (new series)*. 61:177–190.

Mauzerall, D. 1976. Fluorescence and multiple excitations in photosynthetic systems. *Biophys. J.* 16:87–91.

Mourou, G., and W. Knox. 1979. High-power switching with picosecond precision. *Appl. Phys. Lett.* 35:492–495.

Mourou, G., and W. Knox. 1980. A picosecond jitter streak camera. *Appl. Phys. Lett.* 36:623–626.

Nordlund, T. M., and R. S. Knox. 1980. Kinetics of chlorophyllin CPII fluorescence: excitation intensity dependence. *Bull. Am. Phys. Soc.* 25:416–417.

Paillotin, G., C. E. Swenberg, J. Breton, and N. E. Geacintov. 1979. Analysis of picosecond laser-induced fluorescence phenomena in photosynthetic membranes utilizing a master equation approach. *Biophys. J.* 25:513–534.

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.

Searle, G. F. W., J. Barber, G. Porter, and C. J. Tredwell. 1978. Picosecond time-resolved energy transfer in *Porphyridium cruentum*. II. In the isolated light harvesting complex (phycobilisomes). *Biochim. Biophys. Acta.* 501:246–256.

Searle, G. F. W., C. J. Tredwell, J. Barber, and G. Porter. 1979. Picosecond time-resolved fluorescence study of chlorophyll organization and excitation energy distribution in chloroplasts from wild-type barley and a mutant lacking chlorophyll *b*. *Biochim. Biophys. Acta.* 545:496–507.

Searle, G. F. W., and C. J. Tredwell. 1979. Picosecond fluorescence from photosynthetic systems *in vivo*. *In* Chlorophyll Organization and Energy Transfer in Photosynthesis. *Ciba Found. Symp. (new series)*. 61:257–281.

Seibert, M. 1978. Picosecond events and their measurement. *Curr. Top. Bioenerg.* 7:39–73.

Shepanski, J. F., and R. W. Anderson. 1981. Chlorophyll-*a* excited single'-state absorption measured in the picosecond time regime. *Chem. Phys. Lett.* 78:165–173.

Shepanski, J. F., and R. S. Knox. 1981. Circular dichroism and other optical properties of antenna chlorophyll proteins from higher plants. *Israel J. Chem.*. In press.

Snavely, B. B. 1977. Continuous-wave dye lasers. *In* Dye Lasers. F. P. Schaefer, editor. Springer-Verlag, Heidelberg. 86–120.

Stavola, M., G. Mourou, and W. Knox. 1980. Picosecond time delay fluorimetry using a jitter-free streak camera. *Optics Comm.* 34:404–408.

Swenberg, C. E., N. E. Geacintov, and M. Pope. 1976. Molecular quenching of excitons and fluorescence in the photosynthetic unit. *Biophys. J.* 16:1447–1452.

Thornber, J. P., J. P. Markwell, and S. Reinman. 1979. Plant chlorophyll-protein complexes: recent advances. *Photochem. Photobiol.* 29:1205–1216.

Van Metter, R. L. 1977. Excitation energy transfer in the light-harvesting chlorophyll *a/b* protein. *Biochim. Biophys. Acta.* 462:642–658.