PICOSECOND EXCITON ANNIHILATION IN PHOTOSYNTHETIC SYSTEMS

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We have previously reported measurements of fluorescence lifetimes for both photosynthetic systems and chlorophyll solutions using a streak camera technique (1, 2) and picosecond excitation. Our lifetime measurements on antenna systems, however, as well as earlier results obtained using a picosecond resolution optical gate (3–5), indicate somewhat shorter decay times than measured with previous techniques (6–10). Our present experimental investigations of a possible intensity-dependent effect as the cause of these differences are prompted by the recent results of Mauzerall (11). He has shown that the quantum efficiency for the fluorescence emission from *Chlorella decreases* at higher pumping intensities for 7-ns duration excitation pulses, and he has interpreted the decrease in terms of a multitrap model of the photosynthetic unit and exciton-exciton collisions. In order to assess the respective roles that singlet and triplet excitons play in the kinetics of these interactions, it is desirable to repeat these measurements using a much shorter excitation pulse and under conditions comparable to the previously mentioned picosecond experiments. In this letter we report results for the quantum efficiency of *Chlorella* as a function of intensity, but with picosecond excitation. A single 20 ps pulse has been selected from a mode-locked laser pulse train for these measurements. Results show a drop of quantum efficiency with intensity in agreement with results of Mauzerall. Previous picosecond fluorescence lifetime measurements must be reinterpreted in view of this nonlinear optical effect.

In order to investigate the possibility that the somewhat shorter lifetimes obtained by picosecond techniques for antenna systems originate from nonlinear optical effects present at high-energy densities, a different experimental arrangement than we used previously for lifetime measurements is required for quantum efficiency measurements. Previously, all the pulses in a mode-locked pulse train were allowed to excite the sample, and the fluorescence produced by one of the pulses could be examined. When making intensity-dependent quantum efficiency measurements, such a pulse train technique may lead to difficulties in interpretation. For example, pulses exciting the sample prior to the pulse chosen for investigation may populate the reaction centers, leave a residual population of antenna chlorophyll molecules in the triplet state, or otherwise change the system. Thereafter, singlets generated by the pulse to be exam-

ined can interact with residual triplets or the altered state leading to results which are difficult to interpret. Therefore, to prevent confusing interactions due to previous pulses, we have selected a single pulse from a mode-locked Nd:YAG oscillator by means of a longitudinal mode KD*P Pockels cell (12). This single 1060 nm pulse is further amplified ($\frac{3}{8}$-in diam Nd:glass amplifier) before frequency doubling to 530 nm in a potassium dihydrogen phosphate crystal. The 530 nm pulse is propagated over a long delay path to the sample allowing diffraction to smooth spatial inhomogeneities. The beam expands to a diameter of 6 mm, and a 2-mm aperture placed on axis truncates the beam to a known diameter with uniform intensity across its radial profile. A lens images this desired radial profile onto the *Chlorella* sample which is contained in a 1 mm path length cuvette whose transmission has been adjusted to be 50% at 530 nm and whose preparation has been described previously (1, 2). After passing through the aperture, the energy in the pulse is measured accurately with a Laser Precision XR-30 detector and RK-3230 energy meter (Laser Precision Corp., Yorkville, N.Y.). Since the radial profile is accurately known, the beam intensity can be calculated. During data collection, a beam splitter directs a portion of the beam toward a photodiode which has been calibrated against the Laser Precision Corp. units. Filters insure that only 530 nm reaches the photodiode. Fluorescence from the front surface of the sample is detected with an S-1 photomultiplier (RCA 7102; RCA, Harrison, N.J.), equipped with a narrow band pass filter at 700 nm. The laser beam and the fluorescence emission are attenuated with calibrated nonsaturable neutral density filters. Signals from both the photomultiplier monitoring the fluorescence emission intensity and the photodiode monitoring the beam intensity are fed simultaneously into a Tektronix dual-beam oscilloscope (Tektronix, Inc., Beaverton, Ore.) and are photograghed on Polaroid film (Polaroid Corp., Cambridge, Mass.).

Experimental measurements of relative quantum efficiency as a function of energy density are shown for *Chlorella pyrenoidosa* in Fig. 1. The quantum efficiency is relatively constant at low energy densities, but begins to decrease at an energy density of

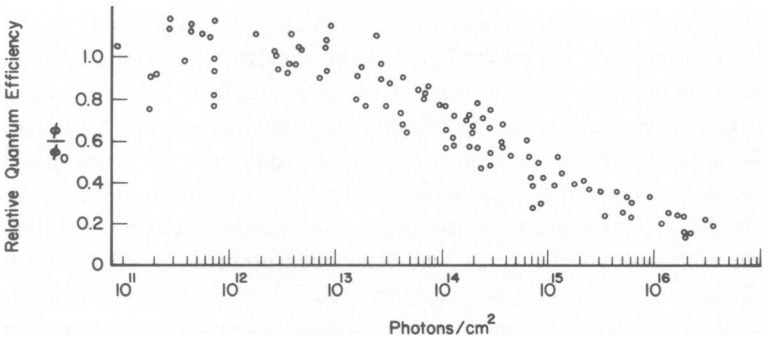

FIGURE 1 Relative quantum efficiency for *Chlorella pyrenoidosa* plotted as a function of photon density. Each circle represents a single laser shot.

about 10^{13} photons/cm^2. At still higher energy densities, the quantum efficiency continues to drop. The energy density represents the actual number of photons which have crossed a given area within the 20 ps pulse.

The experimental quantum efficiency results are consistent with a nonlinear optical effect caused by interactions of the excited state population. This effect would occur when more than one photon excites a photosynthetic unit leading to the simultaneous presence of more than one excited state chlorophyll molecule per photosynthetic unit. Then, excited state singlets can also interact with one another leading to singlet-singlet annihilation, the excess energy possibly disappearing in the form of heat or in the formation of triplets. Since the fluorescence intensity is proportional to the population of excited singlet chlorophyll molecules, the diminution of singlets by such processes would lead to a shortening of the lifetime and a decrease in quantum efficiency. Such interactions have been noted previously by Fröhlich and Mahr (13) in connection with exciton migration in crystals and by Mauzerall (11). In *Chlorella* the total cross section of absorption is thought to be of the order of 100 Å^2 at 530 nm, so that an an energy density of about 10^{13} photons/cm^2 the quantum efficiency should begin to decrease due to "multiple hits" of the photosynthetic units.

It is remarkable that even though our excitation pulse is 350 times shorter than that of Mauzerall (11) the results are so similar. This suggests that the curve for the quantum efficiency as a function of energy density is time-independent for times shorter than 7 ns. Apparently, triplets do not play a major role in single-pulse experiments since several nanoseconds are usually required to build a significant population and this would be inconsistent with our picosecond results. Thus, the singlet-singlet annihilation processes appear to be a prime candidate for explaining the decrease in quantum efficiency. Following Mauzerall, it is essential to introduce a Poisson distribution for the average number of singlets excited per photosynthetic unit as well as an additional stochastic process such as size variations or trap boundary conditions within the photosynthetic units in order to account for the very slow drop of the quantum efficiency with intensity. However, it is very difficult to explain the residual quantum efficiency of 20% observed at high intensities. There are, no doubt, additional competing processes at these intensities. Even so, it appears that much information about the size and structure of photosynthetic units can be obtained from these and future intensity-dependent quantum efficiency experiments.

These results are apparently not influenced by competing intensity-dependent processes which cause increases in quantum efficiency with energy density such has been observed with continuous excitation by Latimer et al. (14). As has been pointed out by Mauzerall (15), these light-induced increases of the fluorescence yield in *Chlorella* are too slow to be primary processes in photosynthesis. For our case, we believe that singlet-singlet annihilation processes explain a decrease in quantum efficiency and a shortening of the lifetime.

All previous fluorescence lifetimes determined using picosecond pulse excitation (1–5) have been made with energy densities which are typically in the 0.1–10 mJ/cm^2

range where nonlinear optical effects play a major role. For example, an energy density of 6×10^{14} photons/cm^2 per pulse is quoted in the experiments of Yu et al. (5), corresponding to about 10 photons absorbed per pulse per photosynthetic unit. From Fig. 1, this is a factor of at least two orders of magnitude too high. Furthermore, interpretation of previous results (1–5) is complicated by the large number of pulses (>100) present in their laser trains. Since triplets, which evolve from singlets by intersystem crossing, live longer than the time interval between pulses in the train, the triplet population would increase from pulse to pulse until a steady-state value is established by triplet-triplet fusion processes. What may then ultimately determine the lifetime with a pulse train are singlet-triplet fusion processes. Rahman and Knox (16) have estimated the singlet-triplet fusion rate to be 6.2×10^{-9} cm^3/s for chlorophyll *a* so that a triplet population density of only 3×10^{18}/cm^3 (about 10% of all chlorophyll *a* antenna molecules) would annihilate the singlet states in about 60 ps. Since this simple estimation is in close agreement with the observed lifetimes, evidence for this mechanism is strong. Conversely, there now seems to be less evidence to support the speculative "dip" model (4).

At present, we find it difficult to measure fluorescence lifetimes below an energy density of 5×10^{13} photons/cm^2 with a streak camera technique and single-pulse excitation. Even at these energy densities, not many photo-electrons are released from the photocathode of the streak tube, so that a statistical analysis of spots formed on a photographic film is required to determine a lifetime. A long-lived component of fluorescence (~0.3–1 ns) is always present in the streak camera data even with single pulse excitation. The short-lived component (~50 ps) observed previously (1–5) should now be interpreted as due to exciton-exciton annihilation, although a short component of about this duration would be present due to photosystem I fluorescence emission (17). The exciton-exciton interpretation is favored because lifetimes determined by the streak camera for many different systems are the same indicating a possible anomaly (A. J. Campillo and S. L. Shapiro, unpublished), and because of these quantum efficiency measurements. In early experiments (1–5) this interaction must dominate because a pulse train was used. It should be noted, however, that these considerations should not affect the interpretation of the results for pigment molecules in solution (1, 2) nor picosecond resolution reaction center experiments (18, 19).

We believe our results lend support to the interpretations and results of Mauzerall (11), and we thank him for informing us of his work, and for helpful discussions.

This work was supported by the Energy Research and Development Administration.

Received for publication 6 October 1975.

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