MULTIPLE EXCITATIONS IN PHOTOSYNTHETIC SYSTEMS

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ABSTRACT The yield of fluorescence in *Chlorella* from a 7 ns pulse of light is found to decrease gradually as a function of the number of hits in the photosynthetic units. The fivefold decrease in yield is spread over some three orders of magnitude of pulse energy and strongly suggests another random process in addition to that of photon absorption. Evidence supports the view that this random process is not in the time but in the spatial domain. The model used to fit the data is that of a unit with multiple traps for the singlet excitation. An excitation is captured by an open trap or destroyed by a filled trap with equal probability. These studies give evidence for the connectivity of the photosynthetic energy transfer apparatus on the short time scale. The short fluorescence lifetimes following picosecond pulse excitation of photosynthetic systems reported by several laboratories may be explained by the effect of multiple excitations.

The excitation of photosynthetic systems with brief, saturating flashes of light has led to much of our detailed knowledge of the photosynthetic systems (Govindjee, 1975; Witt, 1971; Diner and Mauzerall, 1973). Since the saturating pulses necessarily cause multiple hits on the system, the effects of such multiple excitations within the pulse time are of great interest. By use of nanosecond laser pulses changes in quantum yield of fluorescence in *Chlorella* with half-life of 25 ns have been measured (Mauzerall, 1972). A detailed study of these rapid fluorescence transients in *Chlorella* will be published.[1] This note describes the effect of multiple excitations on the fluorescence yield during the short pulse of light. An account of this work was presented at the 5th International Congress of Biophysics (August 1975).

Details of apparatus and procedure are presented elsewhere (Mauzerall, 1972).[1] Essentially a 7 ns (FWHM: full width at half maximum) pulse of 337 nm light from a nitrogen laser is focused on a 5 μl sample of flowing algae. Fluroescence is measured with a gated detector system, using a 12 ns (FWHM) gate time. Linearity is ensured by using the detector in a near-null mode: the detector is attenuated inversely to the intensity of the excitation pulse. A second laser pulse variable in time and intensity is used to determine the fluorescence yield after the main excitation pulse. In the data presented here its integrated intensity is kept below 0.03 hits per unit. Each pulse pair hits a fresh sample of dark adapted algae.

[1] Mauzerall, D. Manuscript in preparation.

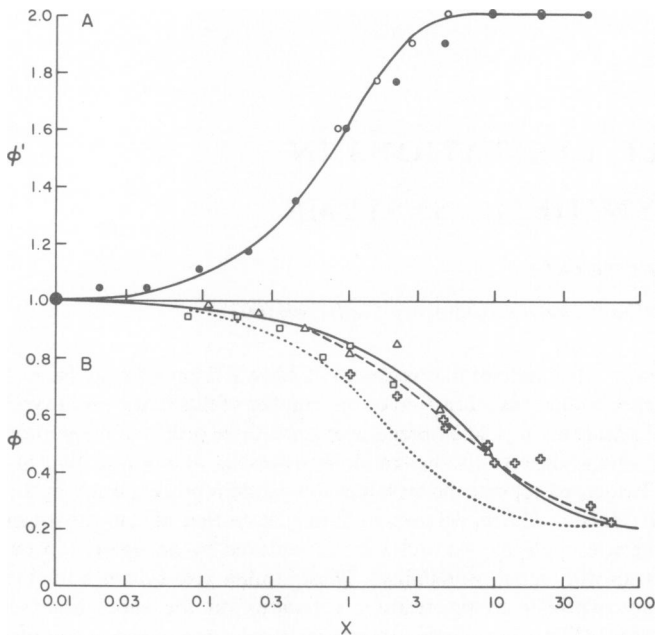

FIGURE 1 The relative quantum yield of fluorescence is plotted vs. average number of hits (X) per unit. (A) ϕ' is the normalized fluorescence yield (•) measured with a low intensity ($X' < 0.03$) 7 ns pulse of light 30 μs following an 7 ns actinic pulse of variable intensity. The open circles (○) are points corrected as discussed in the text, and the solid line is the cumulative one hit Poisson distribution. The fit defines the point $X = 1$. (B) ϕ is the normalized fluorescence yield averaged over the total actinic pulse. The various symbols refer to differing laser powers before attenuation and differing optical filters. The lines represent the behavior of the system following models described in the text. The measured fluorescence emission band was 687 ± 10 nm.

The results are shown in Fig. 1. The top graph (A) shows the normalized increase of quantum yield of fluorescence, ϕ', determined 30 μs after the main pulse as a function of integrated pulse intensity. The random hits of the photosynthetic units are expected to follow the Poisson distributions if the units are separate, and will be very close to this distribution if there is limited overlap. The lower intensity half of the curve follows the single hit cumulative Poisson distribution, but there is significant deviation at higher intensities. Note that the usual arguments in favor of energy transfer between filled and open units in system II (Joliot et al., 1968; Wang and Meyers, 1973) would make the curve sharper, not more shallow. The open circles are points corrected as described below. The abscissa is the average hits per pulse, $X = \sigma \int I dt$, where σ is the optical cross section of a unit, I is the light intensity, and the integral is over the pulse width. The pulse contains about 2×10^{14} photons per cm^2 at $X = 1$, and this corresponds to a photosynthetic unit size of roughly 10^2 chlorophyll molecules. The stamina of *Chlorella* is remarkable: the effects of extreme multiple hitting, 30 hits in 10 ns, have fully decayed by 30 μs.

The lower curve (B) shows that the relative quantum yield of fluorescence during the

main pulse decreases as a function of number of hits. The fluorescence yield has been normalized to that in a very weak pulse. In fact the curve is constant to about one hit per 4×10^4 chlorophylls.[1] The lack of fluorescence increase irrespective of number of hits is consistent with the earlier determination of the finite rise times for this increase (Mauzerall, 1972). The decrease in yield cannot be fit to any simple Poisson distribution. The "single hit" Poisson saturation[1] curve is shown as the dotted line, with 20% of the fluorescence yield assumed constant. This is the curve for the system in which the first hit has a time-independent yield of ϕ_o and the yield of the second and all succeeding hits is zero. The very broad character of the decrease suggests that a second random process is occurring. This could be either in the time or the space domain; let us look at each general possibility in turn.

The most likely random effect in the time domain is the lifetime of a less fluorescent state in the system. The overlap of this random change in yield with the random hits will, in the steady-state limit, produce a broad hyperbolic curve similar to that in the lower part of Fig. 1. We solve the problem in two parts. The single hit units have a yield independent of the time scale. The units having two and greater hits will be hit with a mean frequency n/T where n is the integral hit number and T is the time of the equivalent square wave of the pulse. The fluorescence yield is given by $\phi_n = \phi_o k/[k + (n/T)]$ for a unit hit $n \geq 2$ times, where k is the sum of rate constants of all channels for the decay of the fluorescent state. The observed fluorescence yield ϕ normalized to the yield at single hits (ϕ_o) is then given by

$$\phi = (1/x)[P_1 + \sum_2^\infty nP_n\phi_n], \tag{1}$$

where P_n are the individual Poisson distributions. The data can be fit (Fig. 1, lower solid curve) with $kT = 5$, assuming that 20% of the fluorescence yield is constant. The same result is obtained if this fraction of unique hits has a yield of one and the remaining fraction, those centers hit during the lifetime $1/k$, has a yield of 0.2. A similar calculation, but allowing that one excitation is always preserved following n hits, fits the data with $kT = 3$. Since T in our experiments is 7 ns, $1/k$ is 1.4 or 2.3 ns. This lifetime is in fact close to the fluorescence lifetime (1.7 ns) observed for light saturated photosynthetic systems (Borisov and Il'ina, 1973). However, there are problems with this interpretation. One is the low quantum yield (here taken equal to the single hit state) necessary to fit the data. The yield of the light saturated state is usually two to three times that of the dark adapted state, e.g., in our experiments, 30 μs after the flash ϕ is 2.4 times ϕ_o. Although one can escape this objection, recent measurements on the picosecond time scale also argue against the above interpretation. Because of the very high intensities necessitated by this technique, the problem of multiple excitations is a severe one and must be kept in mind on considering the very fast (≈ 60 ps) lifetimes that have been observed (Seibert and Alfano, 1974; Yu et al., 1975; Kollman et al., 1975). Campillo et al. (1976) have repeated the measurement of Fig. 1 B on the picosecond time scale. They observe a similar very broad decrease of the quantum yield.

The optical cross section at 530 nm is within a factor of two of that at 337 nm. Since Eq. 1 contains the parameter n/T, the 300-fold decrease in T would have reduced the fluorescence yield to the first term of the above equation which gives a curve even sharper than the dotted line of Fig. 1. The contrary result argues strongly that the second random process is not in the time domain.

The simplest distribution in the spatial domain is a variation in σ for the photosynthetic units. However, simple distributions, e.g., half the units with σ and the other half with 0.5 σ, which give a crude fit to Fig. 1 A, fail to fit Fig. 1 B. Results on the σ of the highly fluorescent state (Δ, Mauzerall, 1972)[1] also argues for a more unique σ. The saturation curve for this state is closer to the "Poisson saturation" of the type shown by the dotted line in Fig. 1 B. It is, of course, clear that the value of σ may be an average taken over the ensemble of units which fits the simple curve within experimental error. Although the data could possibly be fit by an arbitrary distribution of σ's a more interesting and useful model presents itself. Suppose there are t traps in each "unit" and that they are equally probable to be visited by an excitation whether they are empty or filled. When the excitation hits an empty trap it fills the trap and on hitting a filled trap it is rapidly degraded. The mechanism could be the well-known rapid decay from doubly excited states in molecules. The probability of new hits for the jth sequential hit on a unit containing t traps is $[(t-1)/t]^{j-1}$ and the fluorescence yield is assumed proportional to new hits. The average fluorescence yield for a unit hit n times is $\phi_n = \phi_o \Sigma_1^n [(t-1)/t]^{j-1}$ and the normalized quantum yield of fluorescence is given by $\phi = (1/x)\Sigma_1^n P_n \phi_n$. The data are fit by this equation with $t = 3$ and assuming that 20% of the fluorescence yield is constant (dashed line, Fig. 1 B). This is equivalent to the assumption that the single hit traps have a yield of one and the multiple hit traps a yield of 0.2. The relation of the postulated multiple traps to photosynthetic reactions remains to be clarified. There is strong evidence that the second hit to a unit produces a long lived triplet state.[1] Multi-trapped units have often been postulated for photosystem II (Clayton, 1967; Joliot et al., 1968; Herron and Mauzerall, 1972; and Wang and Myers, 1973). However, these models attempt to explain the increased oxygen or fluorescence yield over that of singly trapped units on the long time scale: 10^{-5}–10 s. The present model defines the decreasing efficiency with increasing hits to the system on the short time scale (10^{-12}–10^{-8} s). The difference in the models may be related to the interpretation of the 35 ns fluorescence yield risetime (Mauzerall, 1972): the coupling between antenna and traps changes in this time range. The present model allows the data of Fig. 1 A to be corrected for the misses caused by the loss of a fraction of the exictations. The open circles show a good fit to the simple cumulative one hit Poisson distribution when corrected for the misses. Thus our description of the multiple excitations on multi-trapping sites furnishes a coherent explanation of the fivefold decrease in the quantum yield of fluorescence.

The residual yield of 20% of ϕ_o at high excitation is intriguing. Campillo et al. (1976) suggest that exciton annihilation may explain their data. Yu et al. (1975) have argued

that the 60 ps lifetime component that they observe is associated with system I. However, the intensities of the 5 ps pulses, and the fact that a train of about 100 pulses are used, will produce multiple excitations in either photosystem. Our studies (Ballard and Mauzerall, unpublished) show that charge transfer between excited porphyrin type molecules is highly efficient. If the multiple excitations were to produce photoionization of the chlorophyll molecules, charge recombination could produce luminescences of various lifetimes as observed. If the mean trapping and de-trapping time of the excitation were 50 ps, and the lifetime of the excited trap were 200 ps, both the lifetimes and the approximately 1/5 saturated yield would be accounted for. Studies of the effects of multiple excitations appear to be a promising way to obtain information on the topology of the photosynthetic units.

It is a pleasure to recognize the assistance of a National Science Foundation grant, BMS 74-11747, and I thank Doctors S. L. Shapiro and A. J. Campillo for sharing information on their work.

Received for publication 7 October 1975.

REFERENCES

BORISOV, A., W. YU, and M. D. IL'INA. 1973. The fluorescence lifetime and energy migration mechanism in photosystem I of plants. *Biochim. Biophys. Acta.* **305**:364.

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.

CLAYTON, R. K. 1967. An analysis of the relations between fluorescence and photochemistry during photosynthesis. *J. Theor. Biol.* **14**:173.

DINER, B., and D. MAUZERALL. 1973. Feedback controlling oxygen production in a cross reaction between two photosystems in photosynthesis. *Biochim. Biophys. Acta.* **305**:329.

GOVINDJEE, ed. 1975. Bioenergetics of Photosynthesis. Academic Press, Inc., N.Y.

HERRON, H. A., and D. MAUZERALL, 1972. The development of photosynthesis in a greening mutant of *Chlorella* and an analysis of the light saturation curve. *Plant. Physiol.* **50**:141.

JOLIOT, P., A. JOLIOT, and B. KOK. 1968. Analysis of the interactions between the two photosystems in isolated chloroplasts. *Biochim. Biophys. Acta.* **153**:635.

KOLLMAN, V. H., S. L. SHAPIRO, and A. J. CAMPILLO. 1975. Photosynthetic studies with a 10-psec resolution streak camera. *Biochem. Biophys. Res. Commun.* **63**:917.

MAUZERALL, D. 1972. Light-induced fluorescence changes in *Chlorella*, and the primary photoreactions for the production of oxygen. *Proc. Natl. Acad. Sci. U.S.A.* **69**:1358.

SEIBERT, M., and R. R. ALFANO. 1974. Probing photosynthesis on a picosecond time scale. Evidence for photosystem I and photosystem II fluorescence in chloroplasts. *Biophys. J.* **14**:269.

WANG, R. T., and J. MYERS. 1973. Energy transfer between photosynthetic units analysed by flash oxygen yield vs. flash intensity. *Photochem. Photobiol.* **17**:321.

WITT, H. T. 1971. Coupling of quanta, electrons, fields, ions, and phosphorylation in the functional membrane of photosynthesis. *Q. Rev. Biophys.* **4**:365.

YU, W., P. P. 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.