BRIEF COMMUNICATIONS

BIMOLECULAR QUENCHING OF EXCITONS AND FLUORESCENCE IN THE PHOTOSYNTHETIC UNIT

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ABSTRACT The recent results of Campillo et al. and Mauzerall on the quenching of the fluorescence of chlorophyll *a* in *Chlorella pyrenoidosa* as a function of the intensity of the laser excitation pulses are rationalized by applying a model invoking singlet-singlet exciton annihilation.

Recently Campillo et al. (1976*a*) and Mauzerall (1976) have observed that the prompt fluorescence quantum yield of *Chlorella pyrenoidosa* decreases with increasing laser excitation intensity. Although Campillo et al. employed a ~ 20-ps excitation pulse, while Mauzerall utilized a 7-ns laser pulse for excitation, the fluorescence quenching curves (plots of the fluorescence quantum yield (φ) as a function of the total number of photons per pulse) were remarkably similar. In both cases a fivefold decrease in φ was observed as the intensity was varied from ~ 10^{13} to 10^{16} photons cm^{-2}. In this communication we analyze their results in terms of well-known cooperative exciton processes in organic crystals. Our analysis allows us to infer an estimate of the singlet-singlet exciton annihilation rate constant, γ, and to account for the similarity between the quenching curves of Campillo et al. and Mauzerall.

Although triplet excitons can quench singlet excitons, they are not important in the present case involving the quenching of the prompt fluorescence with increasing laser excitation. This may be rationalized as follows: if we adopt the approximate value of 33% for the molecular quantum yield of fluorescence and a radiative lifetime of 15 ns, then the chlorophyll *a* intersystem crossing rate, k_{IS}, is ~ $1.3 \times 10^8 \ s^{-1}$. A triplet population of any significance therefore needs at least ~ 10 ns to build up, even when the quenching of singlet excitons is absent. If the in vivo fluorescence lifetime is of the order of 1 ns, then the efficiency of producing triplet excitons is only ~ 15%, assuming that there is *no* change in k_{IS} in vivo; the efficiency will be reduced even further if the singlet exciton lifetime is still shorter.

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Thus, for the time scales of single pulse laser excitation employed by Mauzerall (1976) and by Campillo et al. (1976*a*), triplet excitons probably play no essential role. To a first-order approximation, therefore, singlet-triplet quenching terms can be omitted from the rate equations governing the evolution of the singlet excitons in the photosynthetic unit under these conditions. It should be stressed, however, that triplet excitons may play an important role when pulse trains are employed, since the triplet lifetime is relatively long, and sufficiently large triplet exciton densities can build up during one pulse and can act as quenchers of singlet excitons produced by the succeeding pulses. It is well known that triplets are effective quenchers of singlets by energy transfer from the singlet to the triplet exciton; in the specific case of chlorophyll *a*, Rahman and Knox (1973) calculated a Förster transfer radius of ~ 48 Å.

The effects of photochemistry are believed to be negligible for the following reason: from measurements of the fluorescence yield, φ, as a function of time, t, after a strong 337.1-nm laser flash (~ 10 ns width, $I_0 = 3 \times 10^{14}$ photons/cm^2), Mauzerall (1972) probed the fluorescence quantum yield with a second, weaker flash, and showed that φ increased for times t up to at least 1 μs. Furthermore, the changes in φ on the time scale of $t < 50$ ns were negligible. Thus there is practically no change in φ due to photochemistry on the short time scales involved in the experiments analyzed here.

If we denote an excited singlet exciton of chlorophyll *a* by S_1, then for a photosynthetic unit containing more than two excitons the following quenching process can occur,

$$S_1 + S_1 \xrightarrow{\gamma} S_0 + S_n^*. \tag{1}$$

Here S_n^* ($n \gtrsim 1$) represents an excited singlet state, which need not correspond to the first electronic manifold, S_0 denotes a chlorophyll *a* molecule in its ground state, and γ is the bimolecular annihilative rate constant. Experimentally this type of process was initially observed in anthracene crystals as a loss in fluorescence intensity as a function of increasing excitation intensity (Tolstoi and Abramov [1967] and Babenko et al. [1971]). Singlet-singlet exciton annihilation also gives rise to intrinsic charge generation in solid anthracene, and it is possible that ions within the bulk chlorophyll molecules are also produced in the photosynthetic unit as a result of singlet-singlet annihilation. These ions can also behave as quenchers of singlet excitons. However, the importance of such processes is difficult to evaluate from the presently available data and therefore they will be neglected here for simplicity. If we set n equal to the number of excitons per unit volume, then the evolution of the singlet exciton density is governed by the rate equation

$$\frac{dn}{dt} = \alpha I(t) - kn - \gamma n^2. \tag{2}$$

The first term denotes the source term whereas k and γ represent all monomolecular and bimolecular rates, respectively, out of the singlet manifold. We have neglected a photon-exciton interaction term since its effects can easily be seen to be negligible.

For picosecond excitation, the source term can be adequately approximated by a

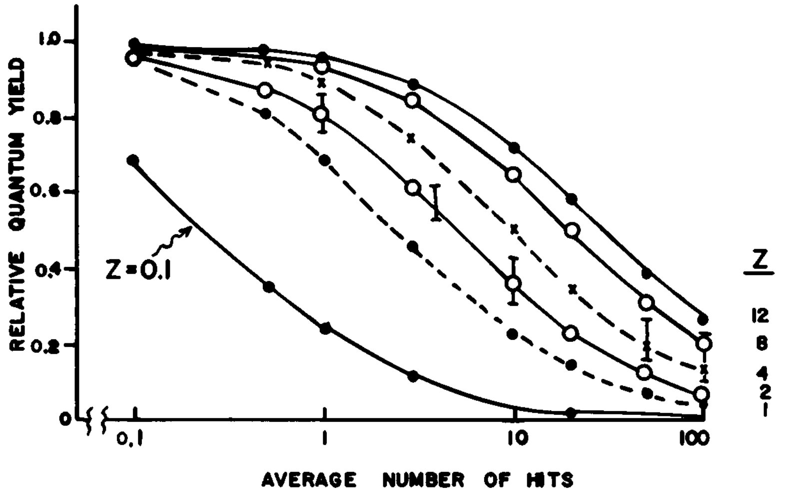

FIGURE 1 The relative quantum yield of fluorescence versus the average number of hits per photosynthetic unit for *Chlorella pyrenoidosa*. Calculated curves were based on a lake model for delta function excitation for several values of $Z = k/G$. Bars denote experimental data of Campillo et al. (1976*a*). One hit has been taken as corresponding to a laser pulse of $I = 2 \times 10^{14}$ photons cm^{-2}.

delta function, and it can be shown that the quantum yield of fluorescence, within the context of the lake model of the photosynthetic unit (Robinson, 1967), is given by the equation

$$\varphi(X) = \int_0^\infty \frac{k_F n(t) dt}{X} = \frac{k_F}{XG} \log\left[1 + \frac{GX}{k}\right], \tag{3}$$

where k_F is the singlet exciton radiative decay rate, and $G = \gamma/V_0$ where V_0 is the volume (area) of the photosynthetic unit. X is a convenient parameter proportional to the light intensity and the volume of the photosynthetic unit V_0; the constant α is defined in such a way that X is the number of hits per ~ 300 chlorophyll molecules (Mauzerall, 1976), i.e.: $X = \alpha I \Delta t\, V_0$ where Δt is the width of the ("square wave") excitation pulse. Even though we have used a lake model approach, it is still convenient to retain the concept of the photosynthetic unit in discussing the number of photons absorbed per flash. In Fig. 1 we plot $\varphi(X)/\varphi(0)$ versus X for several values of $Z = k/G$; this parameter essentially corresponds to the ratio of the monomolecular to the bimolecular decay rate constants.

The data of Campillo et al. (1976*a*) are represented by bars in Fig. 1; the bars indicate the spread in their experimental data. It is readily apparent that favorable agreement is obtained for $Z \approx 2$, implying a bimolecular decay rate of $G \sim (2\tau)^{-1}$, where τ is the in vivo chlorophyll *a* singlet lifetime under conditions of low excitation intensity.

In Mauzerall's experiment, the steady-state solution to Eq. 2 is appropriate due to the long width of the excitation pulse, $\Delta t \sim 7$ ns, and the shortness of the singlet

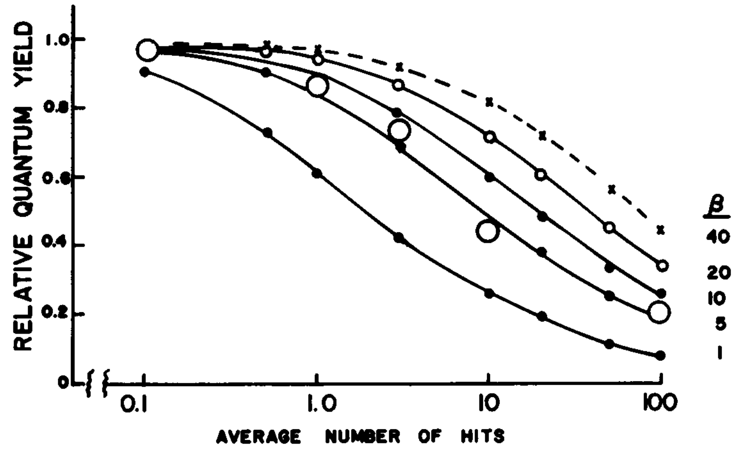

FIGURE 2 The relative quantum yield of fluorescence versus the average number of hits per photosynthetic unit for *Chlorella pyrenoidosa*. We calculated curves for several values of β, assuming steady-state conditions (see text). Large circles denote experimental points reported by Mauzerall (1976).

exciton lifetime, 1 ns or less (Hervo et al. [1975]). Furthermore, since Δt is longer than the fluorescence lifetime, we can make the simplifying assumption that the fluorescence occurs only during the time Δt (i.e. the rise time at the beginning and the fluorescence decay at the end of the pulse are neglected). With these conditions the relative quantum yield of the fluorescence is given by

$$\varphi(X)/\varphi(0) = \frac{1}{X}\left\{-\frac{\beta}{2} + \frac{\beta}{2}\sqrt{1+\frac{4X}{\beta}}\right\}, \qquad (4)$$

where $\beta = k^2 \Delta t/G$. The theoretical curves given in Fig. 2 show that a reasonable fit to Mauzerall's data, represented by the large circles, is obtained for $\beta = 5$. This gives $G \sim \Delta t/5\tau^2$.

The values of the bimolecular annihilation rate constant G, calculated from the theoretical fits to the data of Campillo et al. and Mauzerall, depend on the value of the in vivo fluorescence decay time, τ. Most recently, Campillo et al. (1976*b*) and Hervo et al. (1975) have remeasured τ and found values of 0.6 and 0.8 ns, respectively. Adopting an average value of $\tau \approx 0.7$ ns, we calculate values of $G \sim 3 \times 10^9\ \mathrm{s}^{-1}$ and $G \sim 0.7 \times 10^9\ \mathrm{s}^{-1}$, using the theoretical fits to the data of Mauzerall and Campillo et al., respectively. Thus, within a factor of about four, the same bimolecular annihilation rate is calculated from the two different experiments. This indicates that the simple singlet-singlet annihilation model proposed here can account semi-quantitatively for the fluorescence quenching curves obtained from two different modes of laser excitation.

The bimolecular annihilation rate G, obtained above, can be expressed in the more conventional units of cubic centimeters per second; using a value of the pigment concentration of ~ 0.1 M, which can be calculated from the data of Park and Biggins

(1964) on quantasomes and assuming that there are 300 molecules per photosynthetic unit yields a value of $V_0 = 5 \times 10^{-18}$ cm^3 for the volume occupied by a photosynthetic unit.

Using $\gamma = GV_0$, we find that the bimolecular singlet-singlet annihilation rate constant in the photosynthetic unit is of the order of 10^{-8} cm^3 s^{-1}. (For a two-dimensional photosynthetic unit with a density of one chlorophyll molecule per 400 Å^2, $\gamma \sim 10^{-2}$ cm^2 s^{-1}). This agrees favorably with values of the singlet-singlet annihilation rate constants observed in organic solids (see for example Swenberg and Geacintov [1973]). The value of γ thus estimated corresponds to a singlet diffusion coefficient of $\sim 6 \times 10^{-3}$ cm^2 s^{-1} if a capture radius of 15 Å is assumed, which also implies that the nearest neighbor singlet exciton transfer rate is of the order of 10^{11} s^{-1}.

Although agreement between theory and experiment is favorable and the inferred values of the fundamental molecular parameters are realistic, this in itself does not constitute proof that singlet-singlet annihilation is occurring. However, this mechanism adequately accounts for the observed shapes of the fluorescence quenching curves by a simple kinetic model. Since the excitons are mobile within the photosynthetic membranes, and since exciton annihilation is well documented in other organic systems, there is no doubt that this is an important fluorescence quenching channel in the photosynthetic unit at high light intensities. (Other correlative processes, such as exciton-ion quenching, probably also play a role). It is suggested that mutants without reaction centers or experiments with photosynthetic systems blocked by poisons may be interesting from a point of view of experimental study. There is no doubt that future fluorescence quenching studies using intense laser excitation will allow for a more detailed molecular description of the primary energy transfer processes in the photosynthetic unit.

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