THEORY OF PICOSECOND-LASER-INDUCED FLUORESCENCE FROM HIGHLY EXCITED COMPLEXES WITH SMALL NUMBERS OF CHROMOPHORES

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ABSTRACT The problem of singlet excitation kinetics and dynamics, especially at high excitation intensities, among a small number of chromophores of a given system has been addressed. A specific scheme for the kinetics is suggested and applied to CPII, a small chlorophyll (Chl)*a*/*b* antenna complex the fluorescence lifetime of which has been reported to be independent of excitation intensity over a wide intensity range of picosecond pulses. We have modeled the kinetics from the point of view that Chl*a* molecules in CPII are Förster coupled so that a second excitation received by the group of Chl*a*'s either creates a state with two localized excitons or raises the first one to a doubly excited state. The data on CPII can be understood on the basis of a kinetic model that does not exclude exciton annihilation during the excitation pulse. The implied annihilation rate is consistent with our theoretical estimates of that rate obtained by applying excitation transfer theory to pairs of molecules both initially excited.

INTRODUCTION

One way of extracting information on the exciton transport characteristics of a given system is to observe excitation kinetics at high excitation intensities through fluorescence, either in a time-resolved fashion or by steady-state yield measurements. Excitons created by optical absorption can undergo decay processes and move in a diffusive manner, occasionally coming within a critical distance of one another. Two excitons critically close together may undergo a transition to a state in which there is one exciton or none. This process that is absent at low intensities is known as annihilation. Its normal experimental manifestations are a decrease in quantum yield and a shortening of the lifetime with increasing excitation pulse intensity.

Until the last decade, the excitation annihilation process was mostly of interest in organic molecular crystals (1–5). With the development of fast spectroscopy techniques, primary energy transfer phenomena in biological systems became accessible through time-resolved experiments (6). A study by Paillotin et al. (7) was the first theoretical approach especially designed to investigate singlet exciton kinetics under intense excitation pulses in photosynthetic systems in which no active traps exist. The theory has been extended to include the effects of trapping at active reaction centers (8).

The optically important components of the light-harvesting apparatus of green plants, photosynthetic bacteria, and algae can be isolated and separated (9–16). As such they provide ensembles of small, noninteracting, identical units in which excitons can be studied for small values of N, the number of chromophores that in crystals are of the order of 10^{23}. The possibility of aggregation of such complexes and of subsequent sorting by size makes it reasonable to consider an investigation of the kinetic processes as a function of size in some controllable way. Consequently, one approach to the understanding of the light-harvesting complex (LHC) is to study and understand the segregated components and then attempt to infer the operation of the whole complex.

Some quantum yield and single-pulse, picosecond time-resolved fluorescence measurements on small complexes (consisting of $N = 2, 3, \ldots 10$ chromophores) extracted from systems of general interest to photosynthesis have been carried out by several researchers (17–22). However, the experimental answer to the question of the existence, and, if it exists, the rate of the annihilation process in a fairly small system is not complete. In nearly all picosecond time-resolved experiments, there has been no reported decrease in the fluorescence lifetime in some cases, even under very high excitation intensities. Here, we will be largely concerned with the results of Nordlund and Knox (17), who measured the intensity dependence of the fluorescence decay from CPII, a Chl*a*/*b* complex of 3 Chl*b*'s and 3–4 Chl*a*'s, which is believed to be a part of the LHC associated with the PSII. There was no observed decrease in the lifetime of this complex (3.1 ± 0.3 ns) in the excitation fluence range 10^{14} to 1.4×10^{17} photons/cm^2 of 20 ps, 532 nm excitation pulses. On the other hand, their data on the aggregated form LHC can be explained by annihilation (17). As discussed later, we have decided that there exist no adequate theories to date specifically designed for small-N cases. We therefore have developed a specific scheme stimulated by the one of Paillotin et al. (7).

Using an estimated pairwise annihilation rate found by applying Förster's excitation transfer theory (23) to pairs of excited molecules, the scheme developed is shown to account for the decay of fluorescence intensity from CPII. Both of the schemes suggested in this study, one on the high-density excitation kinetics and one on the theoretical estimate of the pairwise annihilation rate, should be of essential interest for studies of other small complexes.

THE QUALITATIVE PHYSICS OF SIZE EFFECTS

Here we discuss qualitatively the effects that could result by restraining excitation to small domains. We will focus especially on complexes much smaller than the entire photosynthetic unit that contains 100–300 chromophores.

First of all, since as the system gets smaller diffusion of excitons will be inhibited by the boundaries, one expects that higher excitation intensities will be required to build up sufficient pairs of colliding excitons (24). Therefore annihilation, if it happens, is expected at higher intensities as the system gets smaller. On the same grounds, the rate of occurrence of annihilation must depend on the number of chromophores among which the excitation can move. In small systems, the overall annihilation rate γ, defined by Paillotin et al. (7), should approach to the pairwise annihilation rate γ^*, between a pair of excited chromophores. As the system gets larger diffusion will reduce the effectiveness of annihilation. It will be shown in detail later that our estimates for γ^* imply fast singlet-singlet annihilation ($\gamma^* \gtrsim 10^{11}$ s^{-1}) in small Chl*a* units.

The initial number of excited state population that may be prepared during excitation by an intense picosecond pulse that may involve fluences as high as 10^{17} photons/cm^2 in a typical singlet-singlet experiment on LHC can be estimated to range to a significant percentage of the initial absorber density. Therefore, at such high excitation intensities, one expects excited state absorption by individual molecules having reasonably long singlet lifetimes of the order of nanoseconds.

Suppose, for example, a homogenous sample of complexes each containing N weak-coupled chromophores is irradiated. After the first photon is absorbed by a complex, a second photon received may either create a second exciton, thus making annihilation possible, or may hit an already excited molecule, raising it to a doubly excited state. There is an appreciable probability that the latter process will occur, i.e., $\sim 1/N$, under the assumption that the absorption coefficients for the first excited ($S_0 \rightarrow S_1$) and second excited ($S_1 \rightarrow S_2$) states are equal.

FORMULATION OF HIGH-DENSITY KINETICS IN SMALL SYSTEMS

Existing Theories

We mention two of the existing theories on singlet-singlet annihilation that can handle size in some controllable way (5, 7).

The theory by Paillotin et al. (7) defines a domain by the number of excitons it contains. Assuming that excitons disappear by radiative and nonradiative monomolecular and biexcitonic (annihilation) decay processes, it predicts analytic expressions for the quantum yield and fluorescence intensity as a function of excitation intensity in the Pauli Master Equation (PME) formalism. Kenkre's theory (5) solves the same kinetic problem for two initially created excitons in the Generalized Master Equation (GME) formalism and therefore is unique in accounting for transport coherence.

The pulse intensity is one of the factors determining the initial population of excitons. In this respect, Kenkre's theory does not have control over the intensity changes, since it is limited to two initially created excitons. On the other hand, the theory of Paillotin et al. allows one to handle the intensity changes systematically. Both theories are, however, restricted to systems in which excited state absorption can be ignored. Either initial conditions are assumed to be prepared arbitrarily by optical absorption (5) or the excitons are taken to be created by a δ-function pulse (7). However, as we already have discussed, if annihilation happens in a small complex, it is expected to happen fast and may start to take place during the pulse itself (see also reference 18). This fact then requires an explicit consideration of a finite-width source term in a careful theoretical analysis.

Model

In light of the above qualitative discussion, we suggest a model adapted from Paillotin et al. for the interpretation of the fluorescence decay from small systems subjected to high-intensity single excitation pulses. It will be seen that our scheme incorporates the detailed considerations of the pulse characteristics such as intensity, width, and wavelength and explicitly takes into account excited state absorption, as well as systematically handling changes in the size of the system.

For a homogeneous sample of noninteracting units each containing N identical chromophores, under single-pulse optical excitation, we model the excitation and relaxation processes as follows:

(*a*) A square shape light pulse of wavelength λ, intensity I and duration Δt is assumed to be incident.

(*b*) The production of excited singlets occurs at a rate $k_0 = I \cdot \sigma_0(\lambda)$ per ground state chromophore. Here, $\sigma_0(\lambda)$ is the absorption cross section of the ground state.

(*c*) Excited singlets decay to the ground state with a monomolecular rate, k.

(*d*) Optical conversion of excited singlets into doubly excited molecules occurs at a rate $k_1 = I \cdot \sigma_1(\lambda)$ per excited chromophore, where $\sigma_1(\lambda)$ is the absorption cross section of the excited state.

(*e*) Doubly excited molecules, which may undergo autoionization from the state produced in the k_1 process, relax to the ground or first excited singlet state at rates k_{r0}

and k_{r1}, respectively. Although the autoionization of a state may in reality be a charge transfer and thus destroy two molecules, our model assumes for simplicity that only one doubly excited, and therefore temporarily inactive, molecule results.

(f) When a complex contains more than one singlet excitation (say i excitations), annihilation occurs at a rate determined by the diffusion of the excitations, the topology of complex, and a pairwise rate γ^*. For "fast-compact" annihilation we take the total rate proportional to the number of pairwise interactions: $\gamma^* i(i-1)/2$.

(g) Annihilation of i singlets with j doubly excited chromophores occurs, similarly, at a rate $\gamma_d ij$. The result is $i-1$ singlets and j doubly excited chromophores.

(h) Doubly excited chromophores absorb at a rate $k_2 = I \cdot \sigma_2(\lambda)$ per chromophore and may thus passively decrease the fluorescence yield. Since they are assumed to be regenerated immediately after they absorb, this process does not effect the time dependence of the predicted fluorescence.

These kinetic processes are summarized in Scheme I as follows:

$$S_0 \xrightarrow{k_0} S_1$$

$$S_1 \xrightarrow{k} S_0$$

$$S_1 \xrightarrow{k_1} S_2$$

$$S_2 \xrightarrow{k_{r0}\,(k_{r1})} S_0\,(S_1)$$

$$S_1 + S_1 \xrightarrow{\gamma^*} S_0 + S_2$$

$$S_1 + S_2 \xrightarrow{\gamma_d} S_0 + S_2$$

$$S_2 \xrightarrow{k_2} S_n \xrightarrow{\infty} S_2.$$

Scheme I

Master Equation and its Solution

Under the foregoing model assumptions we follow the approach of Paillotin et al. (7) solving a Master equation for population of states (ij), where i and j are the number of singlet excitons and doubly excited molecules, respectively. Whereas Paillotin et al. considered only the singlets, their number ranged to arbitrarily large values. Our pair (ij) ranges over $N' = (N+1)(N+2)/2$ values as may be seen by a simple count, noting that $0 \le i + j \le N$. The probabilities $P_{(ij)}(t)$ that a complex contains i excitations and j doubly excited molecules obey the following PME for the times $t \le \Delta t$,

$$dP_{(ij)}(t)/dt = -\Sigma_{(i'j')} X_{(ij)(i'j')} P_{(i'j')}(t), \qquad (1)$$

where the $N' \times N'$ dimensional matrix X contains all the kinetic information of the above model. The matrix elements of X are governed by the following equation:

$$\begin{aligned} dP_{(ij)}(t)/dt = &-(jk_{r0} + jk_{r1} + ik + \gamma_d ij + [N - i - j]k_0 \\ &+ \tfrac{1}{2}\gamma^* i[i-1] + ik_1)P_{(ij)}(t) \\ &+ k_{r0}(j+i)P_{(ij+1)}(t) + k_{r1}(j+1)P_{(i-1j+1)}(t) \\ &+ k(i+1)P_{(i+1j)}(t) + \gamma_d(i+1)jP_{(i+1j)}(t) \\ &+ k_0(N - [i-1] - j)P_{(i-1j)}(t) \\ &+ k_1(i+1)P_{(i+1j-1)}(t) \\ &+ \tfrac{1}{2}\gamma^*(i+1)(i+2)P_{(i+2j-1)}(t). \end{aligned}$$

The origin of the terms in this equation is illustrated in Fig. 1.

We consider the case in which the complexes are initially unexcited, i.e., $P_{(00)}(0) = 1$ and all other $P_{(ij)}(0)$ are zero. The solutions of Eq. 1 are then

$$P_\alpha(t) = \sum_{\beta=1}^{N'} S_{\alpha\beta} e^{-\lambda_\beta t} S^{-1}_{\beta 1}, \qquad (2)$$

where α is a double-index label that stands for (ij), $\{\lambda_\beta\}$ are the eigenvalues of X, and $\{S_{\alpha\beta}\}$ is the eigenvector of X belonging to eigenvalue λ_β. Eq. 2 is obtained by straightforward matrix algebra, in which we take the following conventions:

$$S^{-1}XS = \Lambda = \begin{pmatrix} \lambda_1 & & & & \\ & \lambda_2 & & & \\ & & \cdot & & \\ & & & \cdot & \\ & & & & \lambda_{N'} \end{pmatrix}. \qquad (3)$$

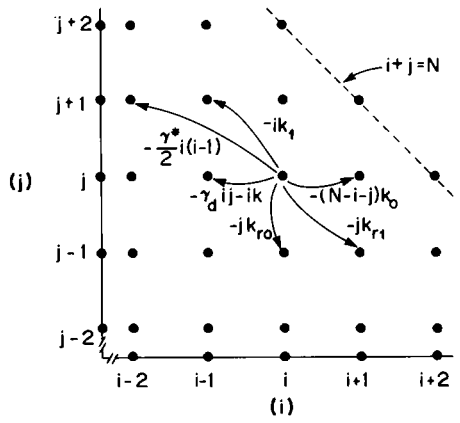

FIGURE 1 Schematic version of part of the X matrix of Eq. 1. Here, i and j are the number of singlet excitons and doubly excited molecules, respectively. There an $N' = (N+1)(N+2)/2$ possible (ij) states satisfying $0 \le i + j \le N$, where N is the number of molecules in the system. Certain states can be created from state (ij) through the kinetic processes defined in the text and as shown by the solid arrows originating with state (ij). Transitions into the state (ij) are not shown for clarity. They would be arrows shifted appropriately such that the tips are at (ij) and the tail at the inverse point if it exists.

At the end of the pulse time Δt, the probabilities will have built up to $P_\alpha(\Delta t) = P_{(ij)}(\Delta t)$ and these values then serve as initial conditions for the relaxation part of the response. For $t \geq \Delta t$ the $P_\alpha(t)$ evolve by the following equation:

$$dP_\alpha(t)/dt = -\sum_{\beta=2}^{N'} Y_{\alpha\beta} P_\beta(t). \quad (4)$$

Y can be obtained from X by setting k_0 and k_1 equal to zero. The first row and column of X must be deleted to prevent the appearance of a zero eigenvalue that plays no role in the fluorescence predictions and that represents the time-dependence of the ultimate relaxed (ground) state.

At time $t \geq \Delta t$ the probabilities will have become

$$P_\alpha(t) = \sum_{\beta,\alpha'=2}^{N'} U_{\alpha\beta} \cdot e^{-\lambda'_\beta \cdot (t-\Delta t)} \cdot U^{-1}{}_{\beta\alpha'} \cdot P_{\alpha'}(\Delta t), \quad (5)$$

where now $\{U_{\alpha\beta}\}$ and $\{\lambda'_\beta\}$ are eigenvectors and eigenvalues of Y, respectively with the convention

$$U^{-1} YU = \Lambda' = \begin{pmatrix} \lambda'_2 & & & & \\ & \lambda'_3 & & & \\ & & \cdot & & \\ & & & \cdot & \\ & & & & \cdot \\ & & & & \lambda'_{N'} \end{pmatrix}. \quad (6)$$

Eqs. 2 and 5 contain all the information needed to compute both the fluorescence yield and the time course of the predicted fluorescence in this model.

Fluorescence Predictions

Except for an overall constant, the instantaneous rate of fluorescence from the model complexes is given by the following weighted sum over the possible states, assuming that the probability of radiation is proportional to $iP_{(ij)}(t)$:

$$j(t) = \begin{cases} \sum_{\alpha=1}^{N'} i \sum_{\beta=1}^{N'} S_{\alpha\beta} \cdot e^{-\lambda_\beta t} S^{-1}_{\beta 1} & \text{for } 0 \leq t \leq \Delta t \quad (7a) \\ \sum_{\alpha=2}^{N'} i \sum_{\beta,\beta'=2}^{N'} U_{\alpha\beta} \cdot e^{-\lambda'_\beta (t-\Delta t)} \cdot U^{-1}_{\beta\beta'} \cdot P_{\beta'}(\Delta t), \text{ for } t \geq \Delta t. & (7b) \end{cases}$$

The total emitted fluorescent energy is thus proportional to

$$J = \int_0^\infty j(t) \cdot dt = \int_0^{\Delta t} (7a) dt + \int_{\Delta t}^\infty (7b) dt. \quad (8)$$

These integrals need not be written out since they are identical to Eqs. 7a and 7b with the replacements

$$e^{-\lambda_\beta \cdot t} \longrightarrow \lambda_\beta^{-1} \cdot [1 - e^{-\lambda_\beta \cdot \Delta t}]$$
$$e^{-\lambda'_\beta (t-\Delta t)} \longrightarrow (\lambda'_\beta)^{-1}.$$

The denominator needed to obtain the quantum yield of fluorescence is obtained by integrating Eq. 2 from 0 to Δt but weighting the various probabilities according to their absorption rather than their emission rates

$$I_{abs} = \sum_{\alpha=1}^{N'} [(N - i - j) k_0 + ik_1 + jk_2] \cdot \sum_{\beta=1}^{N'} S_{\alpha\beta} \cdot ([1 - e^{-\lambda_\beta \cdot \Delta t}]/\lambda_\beta) \cdot S^{-1}_{\beta 1}. \quad (9)$$

Now the quantum yield relative to the monomolecular yield is $\phi = J/I_{abs}$ = Eq. 8/Eq. 9. It appears, therefore, that the problem we have posed is largely solved by matrix manipulation and choice of a reasonably small set of parameters.

ESTIMATION OF SINGLET-SINGLET ANNIHILATION RATES

To estimate the annihilation rates between pairs of excited singlets, we follow the arguments given in regard to singlet-triplet annihilation that have been used for theoretical estimates of the annihilation rates for anthracene, rhodamine, and chlorophyll by Rahman and Knox (25). If the acceptor is already in its excited state before donor loses its energy such that there are two singlet excitations in the system, then upon energy transfer between them the donor goes to its ground state while the acceptor jumps to a higher state. From there it will return to excited state or will drop to ground state. The net result is loss of at least one of singlets i.e., annihilation. Therefore, the pairwise rate of annihilation can be regarded as energy transfer rate between two excited molecules under the assumption that the transition from the doubly excited state to singly excited or ground state happens fast.

The rate for this pairwise annihilation (γ^*) is therefore estimated by applying Förster's resonance transfer theory (23) for the pairwise rate of nonradiative energy transfer between a donor (D) and an acceptor (A) a distance R apart as given by

$$F_{AD} = \frac{1}{\tau_0} \cdot \left(\frac{R_0^{AD}}{R}\right)^6,$$

where τ_0 = the natural radiative lifetime of the donor, and

$$(R_0^{AD})^6 = \frac{9K^2 \cdot c^4}{128 \cdot \pi^5} \cdot \int_0^\infty \frac{f^D(\upsilon) \cdot \sigma_A(\upsilon)}{\upsilon^4 \cdot n(\upsilon)^4} d\upsilon, \quad (10)$$

where K^2 is an orientation factor that equals 2/3 for a random transition dipole distribution, c is the speed of light, υ is frequency, $n(\upsilon)$ is the index of refraction of the medium, $f^D(\upsilon)$ is the donor fluorescence spectrum normalized to one, and $\sigma_A(\upsilon)$ is the acceptor absorption cross-section. R_0^{AD} is the effective radius at which the probability of energy transfer to the acceptor, in our case already excited, equals that of decay by light emission from the donor.

For our application γ^* is given by Eq. 10, where both the donor and acceptor are chlorophyll molecules in their first excited singlet states (A = D = S, singlet). The pairwise

singlet-singlet annihilation rate is $\gamma^* = 1.9 \times 10^{11}\ s^{-1}$ for $R_0^{ss} = 6.7$ nm, $n = 1.4$, $\tau_0 = 15.2$ ns (27), and $R = 2.0$ nm (29). The in vivo value for R_0^{ss} is estimated using the fluorescence spectrum of Chl*a* in ether (28) and the first excited singlet state absorption spectrum of Chl*a* in pyridine (30), both being shifted to approximate Chl*a*'s spectral overlap in the chlorophyll protein. There are two final states possible when energy transfer occurs between two identical molecules in the same state ($S_0^A + S_2^B$ or $S_0^B + S_2^A$), where S_2 is an upper excited state. Therefore, to obtain γ^* we take twice the rate given by Eq. 10.

This result for the pairwise annihilation rate γ^* can also be used to calculate the bimolecular continuum rates γ^{ss} ($cm^3 \cdot s^{-1}$), for large Chl*a* aggregates through the theories by Suna (3) and Yokota and Tanimoto (31) for comparison with experimental results. The bimolecular rate of Chl*a* singlet-singlet annihilation in vivo is $\sim \gamma^{ss} = 13 \times 10^{-9}\ cm^3 \cdot s^{-1}$ using the theory of Yokota and Tanimoto. The formula $\gamma^{ss} = 0.676 \cdot 4\pi \cdot \alpha^{1/4} \cdot (D^*)^{3/4}$, where $\alpha = (R_0^{ss})^6/\tau_0$ and $D^* = D_A + D_D$, with the D_D and D_A donor and acceptor diffusion constants, respectively, is used in the calculation. Suna's theory (3) yields $\gamma_\infty^{ss} = 22 \times 10^{-9}$ $cm^3 \cdot s^{-1}$, where γ_∞^{ss} (diffusion limit of γ^{ss}) $= 8\pi \cdot R \cdot D \cdot (1 + [\beta/D]^{1/2} \cdot R)$ for a three-dimensional system, β is the unimolecular decay rate and R is the average nearest neighbor separation.

γ^{ss} can be expressed in the form $\gamma^{ss} = \gamma_\infty^{ss}/(1 + \gamma_\infty^{ss}/[\gamma^* \cdot v])$, where v is the exclusion volume. For an exclusion volume of 8A in radius, the bimolecular rate of Chl*a* singlet-singlet annihilation in vivo of $\gamma^{ss} = 5 \times 10^{-9}\ cm^3 \cdot s^{-1}$ is found. For the above calculation the Chl*a* singlet exciton diffusion constant is determined by (26) $D = FR^2$, where F = the rate of energy transfer between an S_1 and a S_0 state Chl*a* molecule and R is the average intermolecular distance between Chl*a*'s in vivo. Using Förster theory, $F = 1 \times 10^{11}\ s^{-1}$ for $R_0 = 6.8$ nm, $R = 2.0$ nm (29), and $\tau_0 = 15.2$ ns (27). The in vivo spectral overlap used to calculate R_0 is approximated similar to that for R_0^{ss}. With the same value of R, $D = 4 \times 10^{-7}\ m^2\ s^{-1}$.

Geacintov et al. (29) have studied singlet-singlet annihilation in spinach chloroplasts at temperatures from 300 K to 21 K measuring $\gamma^{ss} = 5\text{–}15 \times 10^{-9}\ cm^3\ s^{-1}$. Nordlund and Knox (17) have measured $\gamma^{ss} = 0.6\text{–}6 \times 10^{-9}\ cm^3\ s^{-1}$ for light harvesting Chl-*a*/*b* protein aggregates. These compare well with the above theoretical results.

RESULTS

Fluorescence decay profiles can be obtained in terms of the proposed kinetic model using Eqs. 7 *a* and 7 *b* for a system of any kinetic characteristics and any given size that we have taken to be $N = 3$ throughout the calculations below, unless otherwise said.

In Fig. 2, the time evolution of the fluorescence intensity is plotted without any annihilation process and for two different values of the annihilation rate that are labeled slow and fast with respect to the pulse width (20 ps) of a typical experiment. At a low excitation intensity, fluorescence decays with the chosen monomolecular decay rate in all three cases. At a high excitation intensity, the decay corresponding to a slow annihilation rate is found to display a nonexponential character that is the expected response from a system in which exciton annihilation happens. However, the ones with no annihilation and with fast annihilation are observed to decay exponentially with nearly the same rates. Any kinetic scheme that does not consider a finite pulse width, thus excluding annihilation and other kinetic processes occurring during the pulse, will always predict a nonexponential decay at early times as the result of annihilation at high excitation intensities. In practice, the fluorescence lifetime may be observed not to decrease with increasing excitation intensity. This is not because annihilation is not occurring, but because annihilation and excited state absorption, which have magnified importance at small N, can start happening during the pulse itself along with all the other kinetic processes described before.

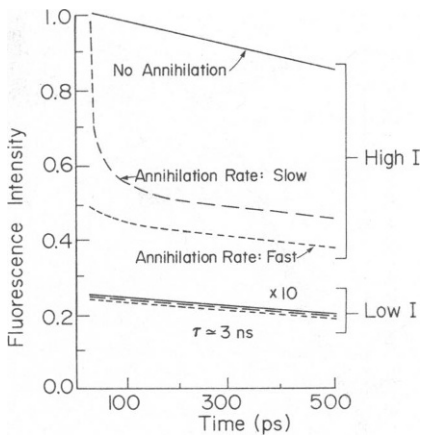

FIGURE 2 Calculated fluorescence profiles at a high (at the top) and a low (at the bottom) excitation intensity for three different annihilation rates. High excitation intensity corresponds to a fluence $\gtrsim 10^{17}$ photons/cm^2 for $\sigma_0(\lambda) \gtrsim 10^{-17}\ cm^2$ and low excitation intensity represents fluences $\sim 5 \times 10^{14}$ photons/cm^2. Here, $k = 3 \times 10^8\ s^{-1}$ and "slow" ($\gamma^* \Delta t = 5 \times 10^{-1}$) and "fast" ($\gamma^* \Delta t = 5 \times 10^2$) are defined with respect to the pulse width of a $\Delta t = 20$ ps square pulse. The absolute value of the exponentially decaying low intensity curves ($\tau \simeq 3$ ns) is magnified 10 times.

The behavior of the fluorescence intensity at the end of a 20 ps square pulse is examined as a function of the excitation intensity (k_0/k) for three different values of the annihilation rate ranging from zero to extremely fast (see Fig. 3). The comparison of the top two curves of Fig. 3 implies that when the annihilation rate is slow the fast component of the fluorescence decay due to annihilation is detectable since it is not fast enough to happen within the duration of the pulse. Whereas, the saturation of the lowest branch (representing an annihilation rate fast compared to the pulse width) at high excitation intensities indicates that annihilation had happened before the excitation pulse ends. In both Figs. 2 and 3, an excited state absorption

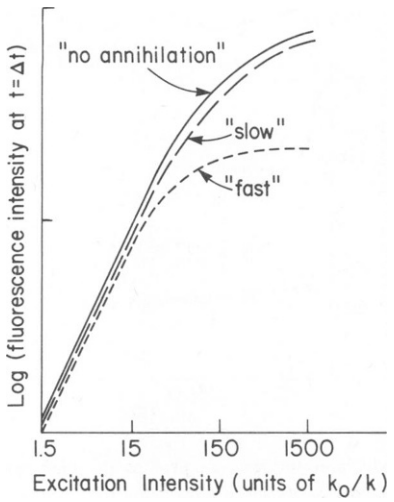

FIGURE 3 Fluorescence intensity at the end of a square pulse vs. excitation intensity for different annihilation rates. The ratio k_0/k represents the excitation intensity through the relationship $k_o = \sigma_o(\lambda).I$. The kinetic parameters used are similar to those of Fig. 2.

three times stronger than that of the ground state as observed in Chl*a* at 532 nm (30), is considered to emphasize annihilation during the pulse width. A fast relaxation of doubly excited states to the ground and singlet states and annihilation between singlets and doubly excited molecules with a rate the same as the singlet-singlet annihilation rate are assumed. The linear dependence of the fluorescence intensity on (k_o/k) at the end of the pulse width for low excitation intensities shows the ineffectiveness of the nonlinear kinetic processes. By observing the kind of effect described in Fig. 3, Kamogawa et al. (18) have deduced that annihilation occurs almost entirely during the pulse in small PSI particles (8–10 *Ch*ls/RC).

The effects of excited-state absorption for different values of the annihilation rate ranging between $3 \times 10^{10}\,s^{-1}$ and $1.6 \times 10^{12}\,s^{-1}$, for the same high intensity value of a 20 ps square pulse are investigated by considering the case with excited state absorption (Fig. 4 *a*) against the case without an excited state absorption (Fig. 4 *b*). The comparison between them is parallel to our physical intuition. If the excited state absorption is excluded, annihilation becomes more observable since there will be more singlet population to participate in this biexcitonic process at any given time.

It was mentioned earlier that the fluorescence decay of CPII has been considered to be exponential ($k = 3 \times 10^8\,s^{-1}$) even under quite intense picosecond pulses, though there may be a small fast component at high fluences (1.4×10^{17} photons/cm^2) as shown by the streak camera traces of Fig. 5 (17).

Consisting of three Chl*b*'s and three to four Chl*a*'s (9, 10), CPII is one of the green plant complexes studied in great detail by optical spectroscopy techniques (32, 33). According to the current hypothesis, three Chl*b*'s of CPII are exciton-coupled and excitation diffuses from site to site among its three or four Förster-coupled Chl*a*'s (32–35). Therefore, our estimate presented in the previous section for the annihilation rate between the pairs of Chl*a*'s can be considered as well a good approximation for the pairwise annihilation rate in CPII. Using this fact the fluorescence decay curves are computed within reasonable values of the other necessary kinetic parameters that are collected in Table I. The superposition of the theoretical fluorescence decay curves on the experimental ones illustrated in Fig. 5 clearly demonstrates that the excitation intensity dependence of the fluorescence decay from CPII can really be understood in terms of a kinetic scheme that explicitly considers pulse characteristics ($\Delta t = 20$ ps, $\lambda_{exc} = 532$ nm, fluence $= I(t)$. $\Delta t = 1.4 \times 10^{16}$ and 1.4×10^{17} photons/cm^2), upper excited state absorption (three times stronger than the ground state absorption) (30) and singlet-singlet

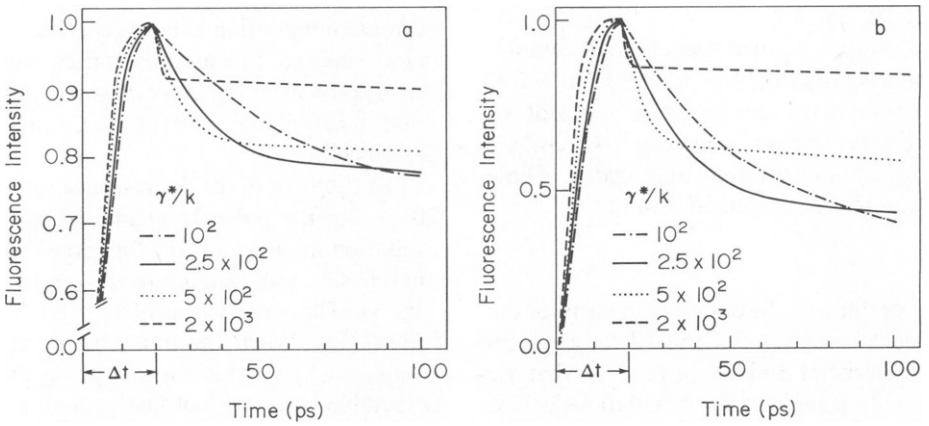

FIGURE 4 Short-time behavior of the fluorescence decay with (*a*) and without (*b*) excited state absorption. Under an excitation fluence $\gtrsim 10^{17}$ photons/cm^2 of $\Delta t = 20$ ps square pulse. The excited state is assumed to absorb three times as well as the ground state that is taken to have a $\sigma_o(\lambda) \gtrsim 10^{-17}$ cm^2 (30). The monomolecular decay rate in terms of which the annihilation rates are expressed is $k = 3 \times 10^8\,s^{-1}$. Depopulation of the first excited and ground states via doubly excited states are assumed to happen with equal rates and faster than any other decay process.

TABLE I
CPII KINETIC PARAMETERS INVOLVED IN EXCITATION KINETICS UNDER INTENSE PICOSECOND PULSES

$k(s^{-1})$*	$k_0(s^{-1})$‡	$k_1(s^{-1})$§	$\gamma^*(s^{-1})$‖
$(3.2 \pm 0.3) \times 10^8$	$(1.2 \pm 0.6) \times 10^{11}$	$(3 \pm 2) \times 10^{11}$	$(2 \pm 1) \times 10^{11}$

*Taken from reference 17

‡Estimated for a ground state absorption cross-section σ_0 (532 nm) – $(2 \pm 1) \times 10^{-17}$ cm^2 (17) assuming that the system is excited by an intense ($\gtrsim 10^{17}$ photons/cm^2) square pulse of Δt – 20 ps using the relationship $k_0 = \sigma_0(\lambda).I$.

§Estimated using the excited state absorption spectrum of Chl*a* in pyridine, which reports that $\sigma_1(\lambda) \simeq (3).\ \sigma_0(\lambda)$ (30) for an excitation pulse with the same characteristics used in estimates of k_0.

‖Estimated as described in the text.

annihilation that occurs with a rate constant ($\gamma^* \geq 10^{11}$ s^{-1}) that is consistent with our theoretical estimate. The only data on the excitation intensity-dependence of the quantum yield of CPII we know of is confined to wide excitation pulses (1 μs) (24). A measurement of this type in the picosecond regime would especially help to determine the depopulation rates of the ground and excited states through the doubly excited state, which we take faster than 1 ps in our fits.

Although so far we have assumed square excitation pulses, this assumption can be relaxed to a Gaussian pulse through simulation by consecutive, narrow square pulses of equal width but with amplitudes correlated to that of a Gaussian of the same half-width as the square pulse still keeping the fluence of the pulse constant. The probabilities of having a particular state in which there are i excitons and j doubly excited molecules at the end of one of such small segments will build up the initial probability of having the same particular state for the next one. An example of such an iteration is depicted in Fig. 6. As expected, the rise is delayed and the shape of the fast decay component is slightly different. In principle, there is no difficulty in improving the simulation by following the argument given above. For better fits to experimental data, a more detailed simulation consisting of more square segments would be necessary. To illustrate the existence of annihilation in a small system and its approximate rate, the square pulse better serves the purpose.

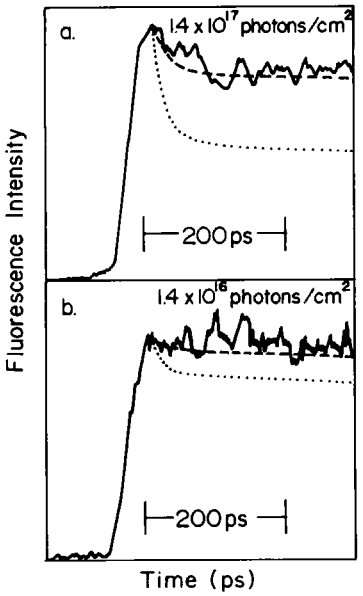

FIGURE 5 Superposition of theoretical fluorescence decay profiles with (——), and without (·····) excited state absorption on the experimental fluorescence decay profiles from spinach CPII (data is taken from [17]). Decay profiles of the similar nature shown in this figure can be obtained for different combinations of the kinetic parameters given in Table I.

DISCUSSION

Although we have applied the kinetic scheme developed to a group of weak-coupled molecules in which a localized excitation is visualized to diffuse from site to site, the strength of coupling is not actually a restriction on the applicability of it. For a system of strong-coupled molecules among which the excitation is delocalized, a second excitation can be viewed simply as exciting the system to a higher and also delocalized exciton state that is broadened by transitions to autoionized states. Since we characterize the system by the number of excitons without assuming any specific interactions between the states, the kinetic scheme is still applicable as long as there is nothing critical about the wavelengths of excitation and emission.

One important element of our interpretation is the use of resonance transfer theory to calculate a rate of energy transfer to an already excited molecule. This is appropriate, since all the transitions being considered are domi-

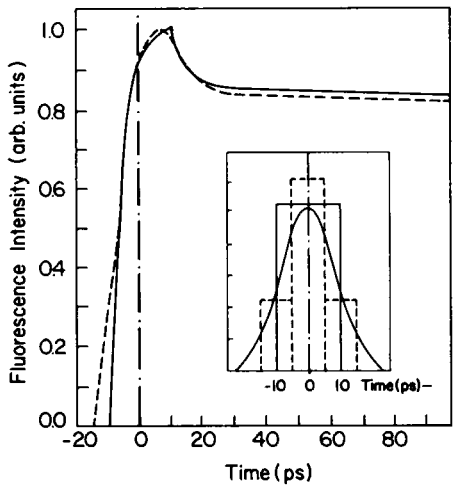

FIGURE 6 Effects of a simulation of a Gaussian pulse via iteration of narrow square pulses on fluorescence decay under high-intensity excitation. The solid line is obtained with a 20 ps square pulse that carries the same fluence of a Gaussian of 20 ps halfwidth (see the insert). The dashed line corresponds to a crude simulation consisting of three square pulse segments as shown in the insert. Here $N = 2$, $\gamma^* = 2.25 \times 10^{11}$ s^{-1} and the other parameters used are $\gamma^* = \gamma_d$, $k = 3 \times 10^8$ s^{-1}, $k_0 = 7 \times 10^{10}$ s^{-1}, $k_1 = 3 \times k_0$, $k_{r0} = k_{r1} = 1.5 \times 10^{12}$ s^{-1}.

nated by incoherent dipole-dipole interactions (28). Our results support a conviction that resonant energy transfer theory is successful in describing singlet-singlet annihilation in chlorophyll systems. The bimolecular theories of Suna (3) and Yokota and Tanimoto (31) have some limitations, yet most of the assumptions made in them are not in conflict with the case of photosynthetic systems, if they are considered as approximations.

The expectation of very short coherence times (< 100 fs) in chlorophyll systems at room temperatures (36) has prompted our choice of a PME formalism to find the evolution of the probabilities in time, thus of the observables. However, we are currently investigating the effects of transport coherence on annihilation using Kenkre's GME theory (5), since developments in subpicosecond pulse generation may make possible observations of coherence effects on annihilation.

In the present calculation, no conversion of singlets to triplets is considered. However, in principle, there is no difficulty extending the scheme. The triad (ijk) would take on $(N + 1)(N + 2)(N + 3)/6$ possible values. As long as one intends to explain the short time behavior ($t < 500$ ps) of the fluorescence decay from a system excited by single picosecond pulses, there is no significant population of triplets.

SUMMARY AND CONCLUSIONS

We have developed a scheme for singlet excitation kinetics and dynamics in highly excited small chromophore complexes that considers pulse characteristics, explicitly takes into account excited state absorption by the chromophores, and handles the size of the complex systematically. We have derived expressions for the fluorescence intensity and the quantum yield that are exact within the model assumptions.

Examples have been given to illustrate that the singlet population at the end of a typical picosecond pulse depends not only on the amount of singlets created by optical absorption, but it is critically determined by the number of doubly excited molecules created through excited state absorption and their conversion rates to singlet and ground state molecules, and by the rate of pairwise annihilation between singlets that can be fast enough to start happening during the pulse itself. We have explained the excitation intensity-dependence of CPII fluorescence decay, which displays a very small fast component at a high fluence, in terms of our scheme with an excited state absorption cross section three times larger than that of the ground states, and with annihilation occurring almost entirely during the pulse with a pairwise rate consistent with our independent theoretical estimate.

We have also shown that the estimated pairwise rate not only explains the fluorescence decay from a small *Chl* complex but also, if utilized in Suna and Yokota and Tanimoto theories, gives the bimolecular continuum rates in agreement with experiments for large chlorophyll aggregates (chloroplasts and LHC). Although, the details are not discussed here, similar conclusions have also been reached for *BChl* complexes. Therefore we are convinced that application of resonance transfer theory to pairs of excited molecules, even though approximate, provides a good estimate of the pairwise rate of singlet-singlet annihilation.

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