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Fluorescence lifetimes in the bipartite model of the photosynthetic apparatus with α, β heterogeneity in photosystem II

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ABSTRACT **Recent studies of the lifetime of fluorescence after picosecond pulse excitation of photosynthetic organisms revealed relatively complex decay kinetics that indicated a sum of three exponential components with lifetimes spanning the range from about 0.1–2.5 ns. These fluorescence lifetime data were examined in the context of a simple photochemical model for photosystem II that was used previously to account for fluorescence yield data obtained during continuous illumination. The model, which consists of a single fluorescing species of antenna chlorophyll and a reaction center, shows that, in general, the decay kinetics after pulse excitation should consist of the sum of two exponential decays. The model also shows that in going from open to closed reaction centers the lifetime of fluorescence may increase much more than the yield of fluorescence and surprisingly long fluorescence lifetimes can be obtained. However, conditions can be stated where fluorescence will decay essentially as a single component and with lifetime changes that are proportional to the yield changes. A heterogeneity was also introduced to distinguish photosystem II_α units, which can transfer excitation energy among themselves but not the photosystem I, and photosystem II_β units, which can transfer energy to photosystem I but not to other photosystem II units. It is proposed that the rather complex fluorescence lifetime data can be accounted for in large part by the simple photochemical model with the α, β heterogeneity in photosystem II.**

Recent advances in picosecond technology have stimulated a number of studies of fluorescence lifetime in photosynthetic organisms in the hope that such investigations will shed new light on the primary processes of photosynthesis. The results have been noteworthy in that they appear to be more complex than had been expected from the photochemical models that were derived primarily from measurements of fluorescence yield. The purpose here is to examine recent fluorescence lifetime data in the context of the earlier bipartite and tripartite models of the photochemical apparatus of photosynthesis.

There is general agreement among the laboratories that use mode-locked lasers to excite fluorescence, photon counting to accumulate the fluorescence decay data, and deconvolution techniques to analyze the decay curves that at least three exponential components are needed to fit the experimental data. These are referred to as the fast component with a lifetime of about 0.10–0.15 ns, a middle component with a lifetime of 0.5–1.2 ns, and a slow component with a lifetime of 1.5–2.5 ns. There is also general agreement that the predominant change in going from the minimum F_0 level of fluorescence, characteristic of a sample with fully open photosystem II (PSII) reaction centers, to the maximum F_M level, characteristic of a sample with completely closed PSII centers (the ratio of F_M/F_0 is generally in the range of 3–5) is in the yield of the slow component. The yield of the slow component may increase by a factor of 20 or more, whereas the lifetime of that component increases by only 50%. The yield and lifetime of the middle component increase by about a factor of 2 and properties of the fast component are more difficult to determine with certainty because the profile of the pulse excitation window has a half-width of 0.2–0.3 ns. In fact, it is generally assumed that the fast component (0.10–0.15 ns) is actually a composite of an even faster decay (0.05–0.08 ns) due to photosystem I (PSI) and a slower component due to the PSII core antenna.

Table 1. Decay components of the fluorescence kinetics in *C. vulgaris*

	Lifetime, ns			Preexponential factor, %			Yield (relative units)			Total yield
	τ_1	τ_2	τ_3	α_1	α_2	α_3	ϕ_1	ϕ_2	ϕ_3	
$F \geq F_0$*	0.13	0.50	1.4	47	52	1	4.0	17.1	0.9	22.0
F_M	0.10	1.2	2.2	12	43	45	0.8	34.0	65.2	100†

All data were calculated on the basis of a triple-exponential model function. At F_M, a biexponential model function is sufficient, however, to describe the experimental decay. The data represent average values from several experiments.

* Decay components calculated from experiments carried out with a photon density of 3.5×10^{11} photons per cm^2.

† Arbitrary reference value.

Data on the yields and lifetimes of the three components from *Chlorella vulgaris* presented by Haehnel *et al.* (1) are shown in Table 1 and in Figs. 1 and 2. Different levels of fluorescence in the range between F_0 and F_M were achieved by adding diuron and hydroxylamine, and particular care was taken to obtain some measurements close to the minimum F_0 level. They reported that the yield of the slow component (τ = 1.4 ns) was negligible at F_0 but increased to represent >60% of the total fluorescence at F_M with a lifetime of 2.2 ns. They also reported that the yield of the fast component decreased substantially during the transition from F_0 to F_M with little change in lifetime (0.13–0.10 ns). The yield and lifetime of the middle component increased but only to a modest extent. The fluorescence decay kinetics at the F_0 and F_M extremes could be fit quite well with double-exponential decay curves but at intermediate levels between F_0 and F_M a triple-exponential decay was required.

The fast component was ascribed to fluorescence from antenna chlorophyll closely associated with PSII reaction centers (the PSII core) with possibly some admixture with PSI fluorescence, the middle component to the light-harvesting chlorophyll a/b complex (LHC), and the slow component to excitons that had visited a closed reaction center and returned to the antenna.

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Abbreviations: PSI and PSII, photosystems I and II, respectively; LHC, light-harvesting chlorophyll a/b complex.

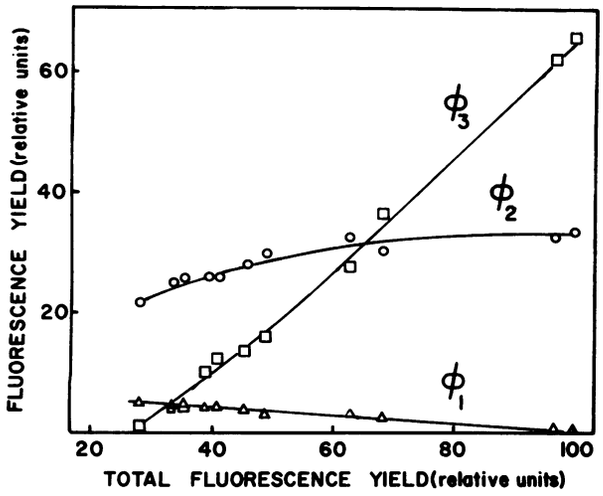

FIG. 1. Yields of the components of the fluorescence kinetics in *C. vulgaris* as a function of the total fluorescence yield. The total fluorescence yield was increased by increasing the concentration of diuron up to a maximum of 20 μM and then by further addition of up to 10 mM hydroxylamine. Addition of diuron alone resulted in a total fluorescence yield of 67%.

The group in Sauer's laboratory at the University of California, Berkeley, had previously obtained similar data for different species (2–4), although they may not have approached the limiting F_0 condition as closely. Their interpretation of the three components was essentially the same, except that they were more explicit in their interpretation of the slow component. They adopted the proposal by Klimov *et al.* (5) that variable yield fluorescence is actually a type of delayed fluorescence that results from charge recombination between P^+, the oxidized reaction center chlorophyll, and I^-, the reduced pheophytin that acts as an electron acceptor prior to the stable primary acceptor. They suggested (2–4) that a quasi-equilibrium is established in closed PSII reaction centers between $P^*{\cdot}I{\cdot}A^-$ and $P^+{\cdot}I^-{\cdot}A^-$ that has a lifetime of several nanoseconds and that this is the origin of the long lifetime of the slow component.

THEORY

Our purpose here is to develop the equations that predict fluorescence yields measured in continuous illumination and the

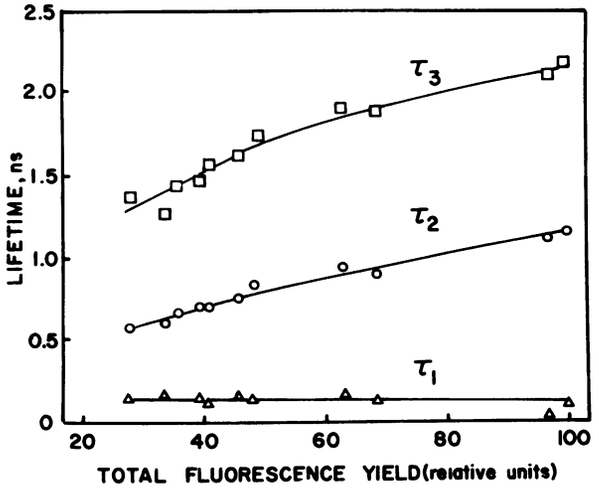

FIG. 2. Lifetimes of the components of the fluorescence kinetics in *C. vulgaris* as a function of the total fluorescence yield. Measurements as in Fig. 1.

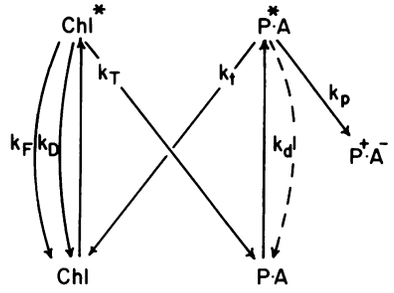

FIG. 3. Photochemical model for PSII consisting of antenna chlorophyll, Chl, the reaction center chlorophyll, P, and the primary electron acceptor, A. The rate constants for energy dissipation and migration are defined in the text.

lifetime data measured by picosecond pulse excitation to see if new elements must be introduced to account for both sets of data. The model for PSII that was used previously in the PSII–PSI bipartite formulation (6, 7) is shown in Fig. 3. This model assumes that LHC is coupled tightly to the PSII core so that there is, in essence, only one fluorescing species of PSII antenna chlorophyll. The excitation energy in the antenna chlorophyll can be dissipated by fluorescence, k_F, nonradiative decay, k_D, or transfer to the reaction center, k_T. Energy in the reaction center chlorophyll can be used for photochemistry, k_p, dissipated by nonradiative decay, k_d, or returned to the antenna, k_t. We assume that fluorescence from the reaction center chlorophyll is negligible.

The differential equations for the steady-state condition under continuous illumination are:

$$d\frac{[\text{Chl}^*]}{dt} = I_a - k_A[\text{Chl}^*] + k_t[\text{P}^*] = 0 \qquad [1]$$

$$d\frac{[\text{P}^*]}{dt} = k_T[\text{Chl}^*] - k_c[\text{P}^*] = 0, \qquad [2]$$

where I_a is the power absorbed by the antenna chlorophyll, k_A is the sum of the dissipative constants for the antenna chlorophyll ($k_A = k_F + k_D + k_T$), and k_c is the sum of the constants for the reaction center chlorophyll ($k_c = k_t + k_d + k_p$). It will prove useful at times to distinguish between open reaction centers, where $k_{c(o)} = k_t + k_d + k_p$, and closed centers, where $k_{c(x)} = k_t + k_d$, because $k_p = 0$ in closed reaction centers.

Eqs. 1 and 2 can be solved for the steady-state concentration of excitons in the antenna, [Chl*], so that the intensity of fluorescence can be determined from $F = [\text{Chl}^*]k_F$. The solution can be obtained for either a separate package model, in which all of the PSII units are separate, or for a matrix model, in which all of the PSII reaction centers reside in one large matrix of antenna chlorophyll. In the separate package model:

$$F = I_a\psi_F\left[\text{A} + \frac{1-\text{A}}{1-\psi_T\psi_{t(x)}}\right].$$

In the matrix model:

$$F = I_a\psi_F\left[\frac{1}{1-\psi_T\psi_{t(x)}(1-\text{A})}\right],$$

where A is the fraction of the PSII reaction centers that are open, $\psi_F = k_F/k_A$, $\psi_T = k_T/k_A$, and $\psi_{t(x)} = k_t/k_{c(x)}$. In this analysis it is assumed that $k_p \gg k_t$ or k_d so that $\psi_{t(o)} = k_t/k_{c(o)} \cong 0$ and $\psi_p = k_p/k_{c(o)} \cong 1.0$. If we consider fluorescence only at the F_0 (A = 1) and F_M (A = 0) levels, the question of energy transfer between PSII units can be avoided because with either

the separate package of the matrix model (6):

$$\frac{F_M}{F_0} = \frac{1 - \psi_T\psi_{t(o)}}{1 - \psi_T\psi_{t(x)}} \cong \frac{1}{1 - \psi_T\psi_{t(x)}}. \quad [3]$$

Also, the yield of photochemistry of PSII at A = 1 can be specified:

$$\Phi_{P_0} = \frac{\psi_T\psi_p}{1 - \psi_T\psi_{t(o)}} \cong \psi_T. \quad [4]$$

Using the same model for PSII (Fig. 3), the differential equations for the distribution of excitons after pulse excitation are:

$$d\frac{[\text{Chl}^*]}{dt} = -k_A[\text{Chl}^*] + k_t[\text{P}^*] \quad [5]$$

$$d\frac{[\text{P}^*]}{dt} = k_T[\text{Chl}^*] - k_c[\text{P}^*]. \quad [6]$$

If we let the initial concentration of excitons in the antenna chlorophyll $[\text{Chl}^*]_0$ be unity at $t = 0$, the simultaneous differential equations can be solved to give:

$$[\text{Chl}^*] = B_1 e^{\lambda_1 t} + B_2 e^{\lambda_2 t} \quad [7]$$

$$[\text{P}^*] = B_3 e^{\lambda_1 t} + B_4 e^{\lambda_2 t}, \quad [8]$$

where

$$\lambda_1 = -1/2\left[k_A + k_c + \sqrt{(k_A - k_c)^2 + 4k_Tk_t}\right]$$

$$\lambda_2 = -1/2\left[k_A + k_c - \sqrt{(k_A - k_c)^2 + 4k_Tk_t}\right]$$

$$B_1 = \frac{k_c + \lambda_1}{\lambda_1 - \lambda_2} \qquad B_2 = -\frac{k_c + \lambda_2}{\lambda_1 - \lambda_2}$$

$$B_3 = \frac{k_T}{\lambda_1 - \lambda_2} \qquad B_4 = -\frac{k_T}{\lambda_1 - \lambda_2}.$$

Expressions for the yields of fluorescence and photochemistry can also be obtained from Eqs. 7 and 8:

$$\Phi_F = \int_0^\infty [\text{Chl}^*]k_F dt = \frac{\psi_F}{1 - \psi_T\psi_{t(o)}} \text{ at A} = 1$$

$$= \frac{\psi_F}{1 - \psi_T\psi_{t(x)}} \text{ at A} = 0$$

$$\Phi_{P_0} = \int_0^\infty [\text{P}^*]k_p dt = \frac{\psi_T\psi_p}{1 - \psi_T\psi_{t(o)}}.$$

Several features of these solutions are worth pointing out:

(*i*) In principle, fluorescence, $F = [\text{Chl}^*]k_F$, will decay as the sum of two exponentials, even though there is only one fluorescing species. In practice, however, one of the two terms may be negligible compared to the other.

(*ii*) There are no imaginary roots for λ_1 or λ_2 because both terms under the square root sign are positive.

(*iii*) The value of the term outside of the square root sign, $k_A + k_c$, is greater than the value of the square root term, so that both λ_1 and λ_2 will be negative. Proof of this statement reduces to the inequality that $\psi_T\psi_t < 1$.

(*iv*) The value of B_1 and B_2 will both be positive for any permissible values of λ_1, λ_2, and k_c, so that both terms in Eq. 7 represent exponential decays. B_3 is negative, indicating an initial rise in the population of P*.

MODEL CALCULATIONS

We can examine the fluorescence decay properties predicted by the model by assuming relative values for some of the rate constants. Initially, we will assume that $k_p = 100\ k_t$, $k_d = 0.25\ k_t$, and $k_T = 0.9\ k_A$. These values are chosen so that the ratio F_M/F_0 is reasonable (3.5), even though the yield of PSII photochemistry, Φ_{P_0}, is quite high (0.90). (If we had assumed that $k_d = 0$, then F_M/F_0 would have had to be 10 to accommodate a Φ_{P_0} of 0.9.) We will assume different values of $k_{c(x)}$ relative to k_A to determine how the decay kinetics depend on the relative value of k_c. For instance, if $k_{c(x)} = 10\ k_A$, then $k_{c(o)} = 81\ k_{c(x)} = 810\ k_A$, $k_t = 0.8\ k_{c(x)} = 8\ k_A$, and $k_T = 0.9\ k_A$. Thus, we can solve for λ_1 and λ_2 in terms of k_A. Table 2 gives values calculated for four cases, the first three of which are case 1,

Table 2. Model calculations

Case			λ_1	λ_2	B_1	B_2	ϕ_1	ϕ_2	τ_{ave}	$(\tau_M/\tau_0)_{ave}$	F_M/F_0
1	$k_{c(x)} = 10\ k_A$ $k_{c(o)} = 810\ k_A$ $k_t = 8\ k_A$ $k_T = 0.9\ k_A$	F_M	$-10.74\ k_A$	$-0.26\ k_A$	0.07	0.93	0.002	0.998	$3.83\ k_A^{-1}$	3.79	3.54
		F_0	$-810.01\ k_A$	$-0.99\ k_A$	1.1×10^{-5}	1.00	1.3×10^{-8}	1.00	$1.01\ k_A^{-1}$		
2	$k_{c(x)} = 1\ k_A$ $k_{c(o)} = 81\ k_A$ $k_t = 0.8\ k_A$ $k_T = 0.9\ k_A$	F_M	$-1.85\ k_A$	$-0.15\ k_A$	0.50	0.50	0.076	0.924	$6.14\ k_A^{-1}$	6.08	3.54
		F_0	$-81.01\ k_A$	$-0.99\ k_A$	1.1×10^{-4}	1.00	1.4×10^{-6}	1.00	$1.01\ k_A^{-1}$		
3	$k_{c(x)} = 0.1\ k_A$ $k_{c(o)} = 8.1\ k_A$ $k_t = 0.08\ k_A$ $k_T = 0.9\ k_A$	F_M	$-1.074\ k_A$	$-0.026\ k_A$	0.93	0.07	0.24	0.76	$29.3\ k_A^{-1}$	29.0	3.54
		F_0	$-8.11\ k_A$	$-0.99\ k_A$	0.001	0.999	2×10^{-4}	1.00	$1.01\ k_A^{-1}$		
4	$k_{c(x)} = 10\ k_A$ $k_{c(o)} = 1{,}010\ k_A$ $k_t = 10\ k_A$ $k_T = 0.9\ k_A$	F_M	$-10.90\ k_A$	$-0.092\ k_A$	0.08	0.92	8×10^{-4}	1.00	$10.90\ k_A^{-1}$	10.8	9.91
		F_0	$-1{,}010\ k_A$	$-0.99\ k_A$	9×10^{-6}	1.00	9×10^{-9}	1.00	$1.01\ k_A^{-1}$		

$k_{c(x)} = 10\ k_A$; case 2, $k_{c(x)} = k_A$; and case 3, $k_{c(x)} = 0.1\ k_A$.

In case 1, it is apparent that the relative yield of the first component [$\phi_i = (B_i/\lambda_i)/\Sigma_i(B_i/\lambda_i)$] is small with respect to the second component at both F_0 and F_M, so that we can consider this case to be essentially a one-component decay that is about 3.8 times slower at F_M than at F_0. In case 3, where $B_1 \gg B_2$ at F_M and $B_2 \gg B_1$ at F_0, we cannot neglect either component. ϕ_1 is negligible at F_0 but the yields of both components are significant at F_M. Case 2, where $B_1 = B_2$ at F_M, is intermediate between case 1 and case 3. We have also calculated the average lifetime for these two component decay curves ($\tau_{ave} = \Sigma_i\phi_i/\lambda_i$). Recall that for all three cases, $F_M/F_0 = 3.54$ and note that in case 3 τ_{ave} increases 29-fold in going from F_0 to F_M. Thus, the model does not predict that the changes in the lifetimes and yields of fluorescence be proportional to one another. However, the proportional relationship will hold to a fair approximation if $k_{c(x)} > 10\ k_A$. We would expect that $k_{c(x)}$ should be greater than $10\ k_A$ because $k_{c(x)}$ is the rate of exciton decay from the reaction center chlorophyll, whereas k_A is the rate of decay from an aggregation of several hundred antenna chlorophylls. If we were to assume that $k_{c(x)} = 100\ k_A$, which is not unreasonable, $(\tau_M/\tau_0)_{ave}$ would agree with F_M/F_0 to within 1%. The conclusion that the lifetime and yield changes do not necessarily track one another may seem somewhat surprising because that has been a generally accepted tenant in photosynthesis mechanisms of photochemistry. The proportional relationship between lifetime and yield is valid for simple quenching mechanisms but does not hold in our photochemical model in which excitation energy can be returned to the antenna from closed reaction centers. The proportional relationship may hold to a close approximation, but if it does, it is only because a particular set of conditions has been met.

Another noteworthy result from Table 2 is that the lifetime of the second component ($\tau_2 = 1/\lambda_2$) at F_M can be surprisingly long (in case 3, 38 times longer than at F_0) without introducing any new elements, such as the Klimov mechanism, into the model. The long lifetime of the λ_2 component of fluorescence at F_M is due to the long lifetime of P*. The lifetime of P* is considerably longer when $k_T > k_t$ (case 3) than when $k_T < k_t$ (case 1). It is also apparent that any process that drains excitons out of P*, such as k_d, should decrease the λ_2 lifetime at F_M. Case 4 in Table 2 makes the same assumptions as case 1, except that k_d is assumed to be zero. As a consequence, $k_t = k_{c(x)} = 10\ k_A$ and $k_{c(o)} = 1{,}010\ k_A$. Again, the yield of the first component is negligible, but the lifetime of the second component at F_M is 2.8 times longer in case 4 than in case 1 due to the quenching effect of k_d in case 1.

We wish to make the case here, for later reference, that higher-than-average ratios of F_M/F_0 can be expected in PSII units that are not in close proximity to PSI and therefore do not transfer excitation energy to PSI. So far as PSII is concerned, energy transfer to PSI is a nonradiative decay process that can be considered to be a part of k_D or k_d. It can be shown (6) that Eq. 3 can also be written as:

$$\frac{F_M}{F_0} = \frac{k_F + k_D + k_T}{k_F + k_D + k_T\,\psi_{d(x)}},$$

where $\psi_{d(x)} = k_d/k_{c(x)}$. It is apparent that for constant values of k_F and k_T, increases of either k_D or k_d will decrease the ratio F_M/F_0. To consider how energy transfer from PSII to PSI might affect k_d, let us assume that the spacial distribution of excitons in the antenna chlorophyll of a PSII unit is different for the photons absorbed from the environment than it is for the excitons transferred from P* back to the antenna. If the excitons returned from P* are, on the average, closer to PSI than are the absorbed excitons, the probability for energy transfer to PSI will be greater for the detrapped excitons. Such an increase in the probability of energy transfer to PSI due to the different spacial distribution of the detrapped excitons will, in effect, change a part of k_t into k_d. Because the ratio F_M/F_0 is very sensitive to k_d, this origin of k_d could play a significant role in limiting the F_M/F_0 ratio of those PSII units that transfer energy to PSI.

BIPARTITE VS. TRIPARTITE MODELS

The above treatment shows that one can obtain a two-component decay of PSII fluorescence from the simple PSII model used in the bipartite model, but it would require a rather delicate balance of the rate constants for both components to be significant. If one adopts the more complex tripartite model (8), in which LHC is assumed to be a separate bed of antenna chlorophyll that can transfer excitation energy via $k_{T(32)}$ to the PSII core antenna, $Chla_{II}$, as well as receive energy from the core, $k_{T(23)}$, the solution to the differential equations will have three roots for [$Chla_{II}$*] and give a triple-exponential decay for fluorescence from the PSII core, $F_{II} = [Chla_{II}^*]k_{FII}$. [LHC*] will also have three roots so that the fluorescence for LHC will also be the sum of three exponentials, $F_{III} = [LHC^*]k_{FIII}$. Thus, in principle, the tripartite model should have sufficient complexity to accommodate the experimental data and, indeed, Nairn used a computer to fit the tripartite model (and the Klimov mechanism) with 12 rate constants to the experimental data in a transition from F_0 to F_M (4). This computer simulation assumed that the fast component was due to fluorescence from the PSII core antenna, that the middle component was due to LHC, and that the slow component was due to charge recombination in closed PSII reaction centers with subsequent exciton migration back to the antenna chlorophyll. However, to obtain a fit between the model and the data, Nairn had to conclude that both $k_{T(23)}$ and $k_{T(32)}$ were $\approx$4 times greater at F_0 than at F_M, whereas it was originally assumed in the tripartite model that these rate constants should be independent of the state of the PSII reaction center (8). However, an even more fundamental concern is that both Nairn (4) and Haehnel *et al.* (1) must assume that LHC is rather loosely coupled to PSII to attribute the middle-lifetime component to LHC, whereas measurements of fluorescence kinetics at −196°C, where fluorescence from LHC and the PSII core antenna can be spectrally resolved at 680 and 695 nm, respectively, showed that LHC was tightly coupled to PSII (9). In fact, it was concluded from those measurements that the simpler bipartite model was adequate for most purposes. Furthermore, any attempt to describe PSII fluorescence in terms of a single homogeneous model ignores the heterogeneity that is generally accepted to occur in PSII.

$PSII_\alpha$ AND $PSII_\beta$

We propose an alternative explanation based on the bipartite model (i.e., Fig. 3) and some assumptions about the heterogeneity of PSII. Melis and Homann (10, 11) proposed that PSII consists of two parts. The α part, which shows a fast rise in the fluorescence induction curve and an inflection near the F_0 level, was attributed to interconnected groups of PSII units that could transfer excitation energy among themselves. The β part, which approaches the F_M level in the induction curve more slowly, was ascribed to individual, separate PSII units that cannot transfer energy to other PSII units. We suggested previously (12) that the fluorescence lifetime data might reflect that type of α, β heterogeneity in PSII. We now make that suggestion more explicit. At F_0, where the yield of the slow component is negligible, we attribute the fast component to $PSII_\alpha$, with some pos-

sible contribution from PSI, and the middle component to $PSII_\beta$. At F_M, where the yield of the fast component appears to be diminished and may represent only PSI, we attribute the middle component to $PSII_\beta$ and the slow component to $PSII_\alpha$. We suggest, using the fluorescence lifetime data of Haehnel *et al.* (Table 1), that in closing the PSII reaction centers, the lifetime of fluorescence from $PSII_\alpha$ increased from 0.13 to 2.2 ns and that the lifetime of $PSII_\beta$ fluorescence increased from 0.5 to 1.2 ns. At intermediate levels between F_0 and F_M, a mixture of three components (fast, middle, and slow) is needed to fit the data with the deconvolution program.

We can also use the data of Haehnel *et al.* to calculate k_A for $PSII_\alpha$ and $PSII_\beta$: $(k_A)_\alpha = (0.13\ \text{ns})^{-1} = 7.7 \times 10^9\ \text{sec}^{-1}$ and $(k_A)_\beta = (0.5\ \text{ns})^{-1} = 2 \times 10^9\ \text{sec}^{-1}$. This value of $(k_A)_\alpha$ may be too large because the 0.13-ns lifetime may represent a combination of a PSI component, expected to be shorter, and a $PSII_\alpha$ component somewhat longer than 0.13 ns. However, if we accept these values for k_A and the *ad hoc* assumptions that $k_{c(x)} = 10\ k_A$ and $k_p = 100\ k_t$, the lifetime for photochemistry (k_p^{-1}) is about 0.2 ps for $PSII_\alpha$ and 0.6 ps for $PSII_\beta$, from which we conclude that k_p^{-1} can be in the order of 1 ps or less.

It has been proposed that $PSII_\alpha$ resides in the interior of the grana stacks, whereas $PSII_\beta$ resides in the peripheral regions of the grana and in the stroma lamellae. PSI is assumed to be in the same general region as $PSII_\beta$. Thus, we would expect significant energy transfer from $PSII_\beta$ to PSI but little or none from $PSII_\alpha$ to PSI and, as a consequence, a larger F_M/F_0 ratio for $PSII_\alpha$ than for $PSII_\beta$. Assuming that $k_{c(x)} > 10\ k_A$, $(\tau_M/\tau_0)_{ave}$ is a fair approximation to F_M/F_0. Again, referring to the data of Haehnel *et al.* in Table 1, we would propose that $(\tau_M/\tau_0)_\alpha = 2.2/0.13 = 16.9$, whereas $(\tau_M/\tau_0)_\beta = 1.2/0.5 = 2.4$. We would suggest that $PSII_\alpha$ is similar to case 4 in Table 2, whereas $PSII_\beta$ is more like case 1. However, the $(\tau_M/\tau_0)_\alpha$ ratio may be too large, again because the lifetime of $PSII_\alpha$ at F_0 may be longer than 0.13 ns and the relatively low value of 2.4 for $(\tau_M/\tau_0)_\beta$ suggests that the quenching by k_d in $PSII_\beta$ should be greater than that assumed in case 1. If case 1 were modified so that $k_t = 0.63\ k_{c(x)}$ instead of $0.8\ k_{c(x)}$ [i.e., by increasing k_d from 0.20 to 0.37 $k_{c(x)}$], still maintaining that $k_{c(x)} = 10\ k_A$, the ratio (τ_M/τ_0) would be decreased to 2.4. The need for such an increase of k_d for the $PSII_\beta$ fraction should not be surprising because we originally assumed that $0.2\ k_{c(x)}$ was an average value of k_d for all of PSII and we now assume, for simplicity, that $k_d = 0$ in the $PSII_\alpha$ fraction.

As an alternative, we might also have proposed that at F_M the middle component was due to $PSII_\alpha$ and the slow component was due to $PSII_\beta$—i.e., that in closing the PSII reaction centers that the fluorescence lifetime of $PSII_\alpha$ increased from 0.13 to 1.2 ns and that of $PSII_\beta$ increased from 0.5 to 2.2 ns. In that case $(\tau_M/\tau_0)_\alpha$ would be 9.2 and $(\tau_M/\tau_0)_\beta$ would be 4.4. Although the choice between these two alternatives is not clear cut, we prefer to attribute the long lifetime component to closed $PSII_\alpha$ units because the lifetime of this component appears to become even longer under conditions that foster greater communication between $PSII_\alpha$ units (3, 4).

If we adopt the α, β heterogeneity and assume that the deconvolution of the fluorescence decay data gives a unique (and correct) solution, we can take the preexponential factors in Table 1 (i.e., α_1 and α_2 at F_0 and α_2 and α_3 at F_M) to be proportional to the cross sections of $PSII_\alpha$ and $PSII_\beta$ at F_0 and F_M. On this basis, the cross sections of $PSII_\alpha$ and $PSII_\beta$ appear to be approximately equal in these data from *Chlorella*. However, the use of the preexponential factors to determine the relative cross sections of $PSII_\alpha$ and $PSII_\beta$ may be pushing the data further than is justified, given the current uncertainties in the methodology in the short time domain as well as some uncertainty as to how close the F_0 and F_M extremes are achieved in some of the picosecond measurements.

Even though the model can predict double-exponential decays for $PSII_\alpha$ or $PSII_\beta$ (or both), we prefer at this point to assume that $k_{c(x)} > 10\ k_A$ for both types of PSII and that both types decay essentially as a single exponential. The double-exponential decays at F_0 and F_M are then due to the mixture of $PSII_\alpha$ and $PSII_\beta$ that obtains. Presumably, the added complexity at intermediate levels between F_0 and F_M (i.e., the triple-exponential decay) is due to the additional mixture of open and closed centers in the $PSII_\alpha$ and $PSII_\beta$ fractions.

The advantages of this scheme are (*i*) it incorporates the α, β heterogeneity of PSII that is known to exert a significant influence on fluorescence; (*ii*) it accounts for both the fluorescence yield and the fluorescence lifetime data without introducing new elements, such as the Klimov mechanism, to account for a long-lifetime component and *ad hoc* assumptions about LHC to account for a middle-lifetime component; (*iii*) it allows us to examine the influence of specific photochemical rate constants on fluorescence lifetimes and yields; and (*iv*) it extends the use of our simple photochemical model for further explorations. The major question at this time is to determine how well this scheme will account for the fluorescence lifetime data at intermediate levels between F_0 and F_M, given reasonable assumptions as to the relative contributions of $PSII_\alpha$ and $PSII_\beta$.

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