Fluorescence lifetimes in the bipartite model of the photosynthetic apparatus with \(\alpha, \beta\) heterogeneity in photosystem II

WARREN L. BUTLER\(^{\dagger}\), DOUGLAS MAGDE\(\ddagger\), AND SYLVIA J. BERENS\(\ddagger\)

\(\dagger\)Department of Biology and \(\ddagger\)Department of Chemistry, University of California at San Diego, La Jolla, CA 92093

Contribution by Warren L. Butler, August 31, 1983

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 I\(_{\alpha}\) units, which can transfer excitation energy among themselves but not the photosystem I, and photosystem I\(_{\beta}\) 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 \(\alpha, \beta\) 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.

Data on the yields and lifetimes of the three components from \textit{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 hydroxyamidine, 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 (\(r = 1.4\) 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 \(F_0\) 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.

| Table 1. Decay components of the fluorescence kinetics in \textit{C. vulgaris} |
|-----------------|-----------------|-----------------|
| Lifetime, ns    | Preexponential factor, % | Yield (relative units) |
| \(r_1\)         | \(r_2\)         | \(r_3\)         | \(a_1\)         | \(a_2\)         | \(a_3\)         | \(\phi_1\)         | \(\phi_2\)         | \(\phi_3\)         | Total yield |
| 0.13            | 0.50            | 1.4             | 47             | 52             | 1               | 4.0             | 17.1           | 0.9           | 22.0         |
| 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 \(3.5 \times 10^{11}\) photons per cm².

* Arbitrary reference value.

Abbreviations: PSI and PSII, photosystems I and II, respectively; LHC, light-harvesting chlorophyll a/b complex.
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 F0 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 pho- phyllin 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*−I−A− and P−I−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 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 PSI–PSI bipartite formulation (6, 7) is shown in Fig. 3. This model assumes that LHC is coupled tightly to the PSII antenna chlorophyll. The excitation energy in the antenna chlorophyll can be dissipated by fluorescence, kF, nonradiative decay, kP, or transfer to the reaction center, kT. Energy in the reaction center chlorophyll can be used for photochemistry, kP, dissipated by nonradiative decay, kD, or returned to the antenna, kT. We assume that fluorescence from the reaction center chlorophyll is negligible.

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

\[ \frac{d[C_{\text{ch}}^*]}{dt} = I_e - k_A [C_{\text{ch}}^*] + k_i [P^*] = 0 \]  

\[ \frac{d[P^*]}{dt} = k_T [C_{\text{ch}}^*] - k_e [P^*] = 0, \]

where \( I_e \) 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 \( k_e \) is the sum of the constants for the reaction center chlorophyll \( k_e = k_i + k_d + k_p \). It will prove useful at times to distinguish between open reaction centers, where \( k_{\text{ch}} = k_i + k_d + k_p \), and closed centers, where \( k_{\text{ch}} = k_i + k_d \), 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, \( [C_{\text{ch}}^*] \), so that the intensity of fluorescence can be determined from \( F = [C_{\text{ch}}^*] k_F \). 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:

\[ F = I_e \psi_F \left[ A + \frac{1 - A}{1 - \psi_T \psi_{\text{ch}} (1 - A)} \right]. \]

In the matrix model:

\[ F = I_e \psi_F \left[ \frac{1}{1 - \psi_T \psi_{\text{ch}} (1 - A)} \right], \]

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 \( \psi_{\text{ch}} = k_i/k_d \). In this analysis it is assumed that \( k_p \gg k_i \) or \( k_d \) so that \( \psi_{\text{ch}} = k_i/k_d \approx 0 \) and \( \psi_p = k_F/k_{\text{ch}} \approx 1.0 \). If we consider fluorescence only at the \( F_0 (A = 1) \) and \( F_A (A = 0) \) levels, the question of energy transfer between PSII units can be avoided because with either

![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%.](image)

![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.](image)

![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.](image)
the separate package of the matrix model (6):

\[
\frac{F_M}{F_0} = \frac{1 - \psi_T \psi_o}{1 - \psi_T \psi_o} \equiv \frac{1}{1 - \psi_T \psi_o}.
\]  

[3]

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

\[
\Phi_F = \frac{\psi_T \psi_o}{1 - \psi_T \psi_o} \equiv \psi_T.
\]  

[4]

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

\[
d \frac{[\text{Chl}^*]}{dt} = -k_A [\text{Chl}^*] + k_f [\text{P}^*]
\]  

[5]

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

[6]

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

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

[7]

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

[8]

where

\[
\lambda_1 = -\frac{1}{2} \left[ k_A + k_c + \sqrt{(k_A - k_c)^2 + 4 k_T k_f} \right]
\]

\[
\lambda_2 = -\frac{1}{2} \left[ k_A + k_c - \sqrt{(k_A - k_c)^2 + 4 k_T k_f} \right]
\]

\[
B_1 = \frac{k_c + \lambda_1}{\lambda_1 - \lambda_2}, \quad B_2 = -\frac{k_c + \lambda_2}{\lambda_1 - \lambda_2}
\]

\[
B_3 = \frac{k_T}{\lambda_1 - \lambda_2}, \quad B_4 = -\frac{k_T}{\lambda_1 - \lambda_2}.
\]

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

\[
\Phi_F = \int_{0}^{\tau} [\text{Chl}^*] k_d dt = \frac{\psi_T}{1 - \psi_T \psi_o} \quad \text{at } A = 1
\]

\[
= \frac{\psi_T}{1 - \psi_T \psi_o} \quad \text{at } A = 0
\]

\[
\Phi_F = \int_{0}^{\tau} [\text{P}^*] k_d dt = \frac{\psi_T \psi_o}{1 - \psi_T \psi_o}.
\]

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 \( \lambda_1 \) or \( \lambda_2 \) 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 \( \lambda_1 \) and \( \lambda_2 \) will be negative. Proof of this statement reduces to the inequality that \( \psi_T \psi_o < 1. \)

(iv) The value of \( B_1 \) and \( B_2 \) will both be positive for any permissible values of \( \lambda_1, \lambda_2, \) 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 \( \text{P}^*. \)

**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 \( k_f = 100 k_A, k_d = 0.25 k_A, \) 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, \( \Phi_{FM} \) 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 \( \Phi_{FM} \) of 0.9.) We will assume different values of \( k_{d(0)} \) relative to \( k_f \) to determine how the decay kinetics depend on the relative value of \( k_c. \) For instance, if \( k_{d(0)} = 10 k_A, \) then \( k_{d(0)} = 81 k_A, k_f = 0.8 k_A, k_T = 0.9 k_A, \) and \( k_d = 0.9 k_A. \) Thus, we can solve for \( \lambda_1 \) and \( \lambda_2 \) 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**

<table>
<thead>
<tr>
<th>Case</th>
<th>( k_{d(0)} )</th>
<th>( k_d )</th>
<th>( k_f )</th>
<th>( k_T )</th>
<th>( B_1 )</th>
<th>( B_2 )</th>
<th>( \phi_1 )</th>
<th>( \phi_2 )</th>
<th>( \tau_{ave} )</th>
<th>( \tau_M/\tau_{ave} )</th>
<th>( F_M/F_0 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>( 10 k_A )</td>
<td>( 810 k_A )</td>
<td>( 810.01 k_A )</td>
<td>( 0.99 k_A )</td>
<td>1.1 ( \times 10^{-5} )</td>
<td>1.3 ( \times 10^{-8} )</td>
<td>1.00</td>
<td>1.01</td>
<td>3.79</td>
<td>3.54</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>( 1 k_A )</td>
<td>( 81 k_A )</td>
<td>( 810 k_A )</td>
<td>( 0.99 k_A )</td>
<td>1.1 ( \times 10^{-4} )</td>
<td>1.4 ( \times 10^{-6} )</td>
<td>1.00</td>
<td>1.01</td>
<td>6.08</td>
<td>3.54</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>( 0.1 k_A )</td>
<td>( 8.1 k_A )</td>
<td>( 810 k_A )</td>
<td>( 0.99 k_A )</td>
<td>1.1 ( \times 10^{-4} )</td>
<td>1.4 ( \times 10^{-6} )</td>
<td>1.00</td>
<td>1.01</td>
<td>29.0</td>
<td>3.54</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>( 10 k_A )</td>
<td>( 1,100 k_A )</td>
<td>( 1,100 k_A )</td>
<td>( 0.99 k_A )</td>
<td>9 ( \times 10^{-6} )</td>
<td>9 ( \times 10^{-6} )</td>
<td>1.00</td>
<td>1.01</td>
<td>10.8</td>
<td>9.91</td>
<td></td>
</tr>
</tbody>
</table>
In case 1, it is apparent that the relative yield of the first component \( \{ \phi_1 = (B_1/\lambda_1)\Sigma_1(B_1/\lambda_1) \} \) 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 \( F_0 \) than at \( F_M \). In case 3, where \( B_1 \approx B_2 \approx B_3 \) at \( F_0 \), we neglect neither component. \( \phi_1 \) is negligible at \( F_M \), but the yields of both components are significant at \( F_0 \). Case 2, where \( B_1 = B_2 = B_{M} \), is intermediate between case 1 and case 3. We have also calculated the average lifetime for these two component decay curves \( \tau_{ave} = \Sigma_1(\phi_1/\lambda_1) \). Recall that for all three cases, \( F_M/F_0 = 3.54 \) and note that in case 3 \( \tau_{ave} \) 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 \( k_{c_0} > 10 k_A \). We would expect that \( k_{c_0} \) should be greater than \( 10 k_A \) because \( k_{c_0} \) 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 \( k_{c_0} = 100 k_A \), which is not unreasonable, \( \tau_{ave} = \Sigma(\phi_1/\lambda_1) \), 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 generally accepted tenet in photobiology 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 \( \tau_2 \) of \( F_M \), 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 \( A_2 \) component of fluorescence at \( F_M \) is due to the long lifetime of \( P^* \). The lifetime of \( P^* \) is considerably longer when \( k_T > k_i \) (case 3) than when \( k_T < k_i \) (case 1). It is also apparent that any process that drains excitons out of \( P^* \), such as \( k_A \), should decrease the \( A_2 \) lifetime at \( F_M \). Case 1 in Table 2 makes the same assumptions as case 1, except that \( k_A \) is assumed to be zero. As a consequence, \( k_i = k_{c_0} = 10 k_A \), and \( \tau_{ave} = 1.010 k_A \). Again, the yield of the first component at \( F_M \) is 2.8 times longer in case 4 than in case 1 due to the quenching effect of \( k_A \), case 1.

We wish to make the case here, for the latter reason, 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 PSI is concerned, energy transfer to PSI is a nonradiative decay process that can be considered to be a part of \( k_D \) or \( k_A \). It can be shown (6) that Eq. 3 can also be written as:

\[
\frac{F_M}{F_0} = \frac{k_f + k_D + k_T}{k_f + k_D + k_T \phi_{c_0}},
\]

where \( \phi_{c_0} = k_A/k_{c_0} \). It is apparent that for constant values of \( k_D \) and \( k_T \), increase or decrease of either \( k_A \) or \( k_D \) will decrease the ratio \( F_M/F_0 \). To consider how energy transfer from PSI to PSI might affect \( k_A \), let us assume that the spatial distribution of excitons in the antenna chlorophyll of a PSI 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 spatial distribution of the detrapped excitons will, in effect, change a part of \( k_A \) into \( k_T \). 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 PSI 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 PSI 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 \( k_{TDT} \) to the PSI core antenna, Chla, as well as receive energy from the core, \( k_{TDO} \), the solution to the differential equations will have three roots for \([\text{Chla}^*]_x\) and give a triple-exponential decay for fluorescence from the PSI core antenna, \( F_{PSII} = [\text{Chla}^*]_x/t_f \). [LHC*] will also have three roots so that the fluorescence for LHC will also be the sum of three exponentials, \( F_{LHC} = [\text{LHC}^*]_x/k_{TDO} \). 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 PSI core antenna, that the middle component was due to LHC, and that the slow component was due to charge recombination in closed PSI reaction centers with subsequent excitation migration back to the antenna chlorophyll. However, to obtain a fit between the model and the data, Nairn had to conclude that both \( k_{TDT} \) and \( k_{TDO} \) were \( 4 \times 10^4 \) times greater than \( F_0 \) 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 PSI 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 PSI to attribute the middle-lifetime component to LHC, whereas measurements of fluorescence kinetics at \(-196^\circ C\), where fluorescence from LHC and the PSI core antenna can be spectrally resolved at 680 and 695 nm, respectively, showed that LHC was tightly coupled to PSI (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* and PSI**

We propose an alternative explanation based on the bipartite model (i.e., Fig. 3) and some assumptions about the heterogeneity of PSI. Melis and Homann (10, 11) proposed that PSII consists of two parts. The \( \alpha \) 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 PSI units that could transfer excitation energy among themselves. The \( \beta \) part, which approaches the \( F_0 \) level in the induction curve more slowly, was ascribed to individual, separate PSI units that cannot transfer energy to other PSI units. We suggested previously (12) that the fluorescence lifetime data might reflect that type of \( \alpha, \beta \) heterogeneity in PSI. 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 PSI*I, with some pos-
At FM, where the yield of the fast component appears to be diminished and may represent only PSI, we attribute the middle component to PSIIa and the slow component to PSIIp. 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 PSIIa increased from 0.13 to 2.2 ns and that the lifetime of PSIIp fluorescence increased from 0.5 to 1.2 ns. At intermediate levels between F0 and Fm, 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 kₐ for PSIIa and PSIIp: (kₐ)ₐ = (0.13 ns)⁻¹ = 7.7 × 10⁶ sec⁻¹ and (kₐ)ₚ = (0.5 ns)⁻¹ = 2 × 10⁶ sec⁻¹. This value of (kₐ)ₐ may be too large because the 0.13 ns lifetime may represent a combination of a PSI component, expected to be shorter, and a PSIIa component somewhat longer than 0.13 ns. However, if we accept these values for kₐ and the ad hoc assumptions that (k₋ₐ) = 10 kₐ and k₋ₚ = 100 kₐ, the lifetime for photochemistry k₋ is about 0.2 ps for PSIIa and 0.6 ps for PSIIp, from which we conclude that k₋ can be in the order of 1 ps or less.

It has been proposed that PSIIa resides in the interior of the grana stacks, whereas PSIIp resides in the peripheral regions of the grana and in the stroma lamellae. PSI is assumed to be in the same general region as PSIIp. Thus, we would expect significant energy transfer from PSI to PSII a little or none from PSII to PSI and, as a consequence, a larger Fm/F₀ ratio for PSIIa than for PSIIp. Assuming that k₋ₐ > 10 kₐ, (τ₋ₐ/τ₋)ave is a fair approximation to Fm/F₀. Again, referring to the data of Haehnel et al. in Table 1, we would propose that (τ₋ₐ/τ₋)ave = 2.2/0.13 = 16.9, whereas (τ₋ₐ/τ₋)ave = 1.2/0.5 = 2.4. We would suggest that PSIIa is similar to case 4 in Table 2, whereas PSIIp is more like case 1. However, the (τ₋ₐ/τ₋)ave ratio may be too large, again because the lifetime of PSI at F₀ may be longer than 0.13 ns and the relatively low value of 2.4 for (τ₋ₐ/τ₋)ave suggests that the quenching by k₋ₐ in PSIIp should be greater than that assumed in case 1. If case 1 were modified so that k₋ₐ = 0.63 k₋ₐ instead of 0.8 k₋ₐ [i.e., by increasing k₋ₐ from 0.2 to 0.37 k₋ₐ], still maintaining that k₋ₐ = 10 kₐ, the ratio (τ₋ₐ/τ₋)ave would be decreased to 2.4. The need for such an increase of k₋ₐ for the PSIIa fraction should not be surprising because we originally assumed that 0.2 k₋ₐ was an average value of k₋ₐ for all of PSII and we now assume, for simplicity, that k₋ₐ = 0 in the PSIIa fraction.

As an alternative, we might also have proposed that at F₉ the middle component was due to PSIIa and the slow component was due to PSIIp—i.e., that in closing the PSII reaction centers that the fluorescence lifetime of PSIIa increased from 0.13 to 1.2 ns and that of PSIIp increased from 0.5 to 2.2 ns. In that case (τ₋ₐ/τ₋)ave would be 9.2 and (τ₋ₐ/τ₋)ave 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 PSIIa units because the lifetime of this component appears to become even longer under conditions that foster greater communication between PSIIa 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., α₁ and α₂ at F₀ and α₃ and α₄ at F₉) to be proportional to the cross sections of PSIIa and PSIIp at F₀ and F₉. On this basis, the cross sections of PSIIa and PSIIp appear to be approximately equal in these data from Chlorella. However, the use of the preexponential factors to determine the relative cross sections of PSIIa and PSIIp 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 F₀ and F₉ extremes are achieved in some of the picosecond measurements.

Even though the model can predict double-exponential decays for PSIIa or PSIIp (or both), we prefer at this point to assume that k₋ₐ > 10 k₋ₐ for both types of PSII and that both types decay essentially as a single exponential. The double-exponential decays at F₀ and F₉ are then due to the mixture of PSIIa and PSIIp that obtains. Presumably, the added complexity at intermediate levels between F₀ and F₉ (i.e., the triple-exponential decay) is due to the additional mixture of open and closed centers in the PSIIa and PSIIp 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 Klino7 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 F₀ and F₉, given reasonable assumptions as to the relative contributions of PSIIa and PSIIp.