Does Slow Energy Transfer Limit the Observed Time Constant for Radical Pair Formation in Photosystem II Reaction Centers?†

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ABSTRACT: We have used spectrally photoselective femtosecond transient absorption spectroscopy on photosystem II reaction centers to show that there are at least two pools of chlorin molecules/states which can transfer excitation energy to P680, the primary electron donor in photosystem II. It has previously been shown that one chlorin pool equilibrates with P680 in 100 fs [Durrant et al. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89,* 11632−11636], and we report here the observation of energy transfer from a second more weakly coupled chlorin pool. The effect of the weakly coupled pool is to increase the apparent time constant for radical pair formation from 21 ps when P680 is selectively excited to 27 ps when the accessory chlorins are excited. We conclude that it is possible to observe both radical pair formation somewhat slowed by an energy transfer step and radical pair formation not limited by this slow energy transfer, depending upon which chromophores are initially excited. These observations provide evidence that when using photoselective excitation of P680, the observed 21 ps time constant for radical pair formation is not limited by a slow energy transfer step.

Conversion of solar energy by the reaction center of photosystem II (PS II)[1] produces the driving force for water splitting by higher plants. Excitation energy is converted into a transmembrane electrochemical potential at the reaction center by an unknown number of electron transfer reactions. The most widely examined reaction center which can be isolated from PS II typically contains six chlorophyll *a* molecules, two pheophytin *a* molecules (Ph), two β-carotenes, and one cytochrome *b*-559 (Gounaris et al., 1990; Kobayashi et al., 1990), having lost all quinones during the isolation procedure. The primary electron donor of PS II is associated with a spectral feature at 680 nm, and is referred to as P680 (van Gorkom et al., 1975; Doring et al., 1969). A pheophytin molecule is known to act as an electron acceptor (Klimov et al., 1977). The secondary electron transfer steps of quinone reduction and tyrosine oxidation do not occur in isolated PS II reaction centers, and the photochemistry of this complex is therefore restricted to formation of the radical pair state $P680^+Ph^-$, and a variety of recombination reactions which follow the formation of this radical pair [reviewed in Seibert (1993)].

Analogies have been drawn between the PS II reaction center and reaction centers of purple bacteria on the basis of the homology of their protein sequences (Michel & Deisenhofer, 1988; Barber, 1987) and comparisons of their acceptor side electron transfer pathways (Rutherford, 1986); their energy transfer properties, however, should be quite different. For example, the primary donor in *Rhodobacter sphaeroides* reaction centers has an excited singlet electronic state which is 150 meV lower in energy than the next optically accessible state of the reaction center, while in PS II reaction centers there are excited states which are separated from excited P680 by less than 1 k_BT at room temperature. There may even be excited states which are isoenergetic with excited P680 (Tang et al., 1990; Kwa et al., 1992; Kwa, 1993). This means that while energy transfer in purple bacterial reaction centers is found to be largely unidirectional toward P870 (Breton et al., 1986), energy transfer in PS II reaction centers might be expected to be extensively bidirectional.

Forward and reverse energy transfer steps have recently been observed in PS II reaction centers using femtosecond transient absorption spectroscopy (Durrant et al., 1992b). This was achieved by photoselecting either a pool of chlorin states/molecules centered at 670 nm (C670 chlorins) or a pool of states centered at 680 nm (C680 chlorins), and observing that equilibration of the excitation energy occurs with a time constant of 100 fs in either case. Although one pair of forward and reverse energy transfers has been identified, many more energy transfer processes must exist, as in principle there will be forward and reverse steps between each of the chlorin pigments/states of the PS II reaction center.

There have been several reports of relatively slow kinetics (tens of picoseconds) in PS II reaction centers which have been assigned to energy transfer from pheophytin and accessory chlorophylls to P680. Wasielewski and co-workers reported the observation of ~25 ps components in transient absorption experiments at 15 and 297 K, which they assigned to energy transfer processes not associated with charge separation (Wasielewski et al., 1989b; Seibert et al., 1992). Tang et al. (1990) concluded from hole-burning spectroscopy that at 7 K energy transfer from accessory chlorophylls to pheophytin occurred with a 12 ps time constant, and from pheophytin to P680 with a 50 ps time constant. Holzwarth and co-workers, studying PS II reaction centers at temperatures between 20 and 277 K, have assigned several fluores-

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[1] Abbreviations: PS II, photosystem II; Ph, pheophytin; P680, primary electron donor of PS II.

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cence components with lifetimes between 13 and 70 ps to energy transient processes (Roelofs et al., 1991, 1993). Recently, Schelvis et al. (1994) have used transient absorption spectroscopy at 277 K to observe a 30 ps component; this component was observed using selective excitation at 662 nm, but was not seen when 687 nm excitation was used and was therefore assigned to an energy transfer process. Slower components of hundreds of picoseconds have also been resolved and assigned to slow energy transfer processes, damaged reaction centers, and/or radical pair relaxations (Hastings et al., 1992; Roelofs et al., 1991; Booth et al., 1991).

Whether these slow energy transfer processes limit the observed time constant for charge separation in isolated PS II reaction centers is currently a matter of debate. Several groups have reported that the observed time constant for charge separation in PS II reaction centers is approximately 3 ps at 277 K (Wasielewski et al., 1989a; Roelofs et al., 1991; Gatzen et al., 1992; Schelvis et al., 1994), shortening to 1.4 ps at 15 K (Wasielewski et al., 1989b; Jankoviak et al., 1989). This is in disagreement with two recent studies which have concluded that the primary radical pair state $P680^+Ph^-$ is produced primarily with a 21 ps time constant at 295 K even when P680 was directly excited (Hastings et al., 1992; Durrant et al., 1993). Another study has reported an average trapping time constant in PS II reaction centers of 13 ± 3 ps (Freiberg et al., 1994), consistent with the lifetime of a transient absorption component observed by McCauley et al. (1992).

It is essential to determine the influence of both the subpicosecond and any slower energy transfer processes upon the observed time constant for charge separation in PS II reaction centers. In this paper, we confirm that although some chlorins equilibrate with P680 in 100 fs, there is at least one chlorin that exhibits slower energy transfer to P680. Moreover, we demonstrate that despite the existence of a slow energy transfer step between some of the pigments in PS II, this slow energy transfer is not the reason for the slow electrogenerative reactions in isolated PS II reaction centers under conditions where P680 is photoselectively excited. A somewhat counterintuitive result, however, is that the fast equilibration of excitation energy previously reported may well play some role in increasing the apparent time constant of pheophytin reduction and P680 oxidation (Durrant et al., 1992b).

MATERIALS AND METHODS

PS II reaction centers were isolated as described previously (Booth et al., 1991; Chapman et al., 1991). Experiments were performed at 295 K as previously described (Durrant et al., 1993), but with one significant difference. In this paper, we present data which were collected using a home-built multichannel diode array detector as well as data collected using a single-wavelength detector. The multichannel detector comprises the following. The output from 2 linear 128 element diode arrays is digitized to 14 bit precision at a total frequency of 1.6 MHz to give a frame rate of 6.5 kHz for each array. One array detects the intensity of the probe beam, while the other records intensity fluctuations via a reference arm of the spectrometer. The outputs are averaged and preprocessed in a home-built controller/digital signal processor along with the output from a single diode which detects intensity fluctuations in the excitation beam, before being output to a PC. The dynamic range of the multichannel detector is 10^6 at 1 Hz bandwidth when used in conjunction with our 6.5 kHz laser system. Data were collected with the polarization of the pump and probe beams either parallel or at the magic angle.

The multichannel detector was set to a resolution of 0.7 nm/pixel to give 90 nm full range across the array and was calibrated using multiple lines from a neon or mercury lamp.

Transient absorption spectra were recorded as a function of time at 13, 66, and 462 fs intervals over 0−2.5, 0−12, and 0−90 ps ranges and fit globally to the following function:

$$\Delta OD(\lambda,t) = \sum_i \Delta OD_i(\lambda) \exp(-t/\tau_i) \qquad (1)$$

where $\Delta OD_i(\lambda,t)$ is the transient absorption change as a function of time and wavelength, $\Delta OD_i(\lambda)$ are the preexponential amplitudes as a function of wavelength (often called kinetic spectra or decay-associated spectra), and τ_i are exponential time constants. $\Delta OD_i(\lambda)$ and τ_i are fitting parameters. The amplitudes are allowed to vary freely at each probe wavelength; the time constants are constrained to be the same at all probe wavelengths. Single-channel data were analyzed as previously (e.g., Hastings et al., 1992). As previously, the data were analyzed using the minimum number of components necessary to obtain random residuals. In all cases, the experiments were repeated a number of times in order to assess the reproducibility of the data and to establish the precision of the results. The experimental spreads quoted in this paper are one standard error (standard deviation divided by $\sqrt{N}$) of the results of N independent fits to different datasets. The spread in our data is highly reproducible from day to day and sample to sample. This allows us to average data in a rigorous manner. The results shown in this paper were produced by signal-averaging for 0.2−1 s per time point. Reaction center sample optical densities were 0.8−1.0 at 675.7 ± 0.3 nm. Experimental conditions were adjusted to obtain a maximum absorbance change of 0.02 at 680 nm in the radical pair spectrum at 100 ps time delay. The spectra in Figures 3 and 4 were normalized against the radical pair spectrum (Durrant et al., 1993).

It has previously been shown that it is possible to excite selectively either of two chlorin pools in the PS II reaction center (Durrant et al., 1992b). The two pools are named C680 and C670. The C680 chlorin pool includes P680, while the C670 chlorin pool includes some or all of the "accessory" chlorophylls. This excitation selectivity was achieved by using spectrally broad excitation pulses centered at 694 and 665 nm, which overlap the red and blue sides, respectively, of the reaction centers' Q_y absorption bands. Spectra of these excitation pulses are shown in Figure 1. The pulses were obtained by amplification of a femtosecond continuum using either Oxazine 720 or Nile Blue as the amplifier dye. The study reported here employed these photoselective conditions in order to examine any differences in radical pair formation in greater detail.

RESULTS

Some of the data in this paper are presented as kinetic spectra which are the amplitudes $\Delta OD_i(\lambda)$ of exponential components. This form of presentation has been chosen when the amplitudes of the kinetic components are much smaller than the overall transient absorption change. In

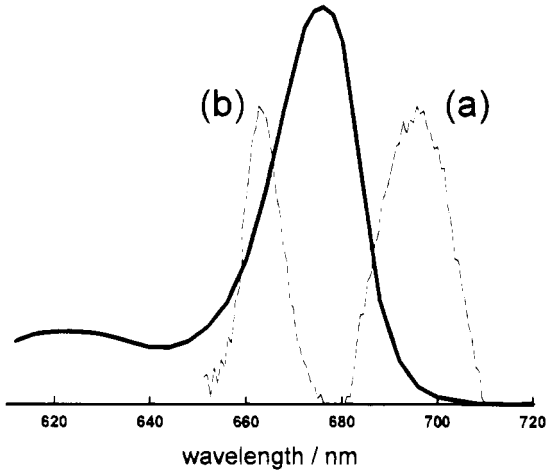

FIGURE 1: Overlap of the reaction center Q_y absorption band (solid line) with the spectra of the excitation pulses used for the C680 (a) and C670 (b) photoselective experiments.

particular, the 21 and 27 ps components in the chlorin Q_y absorption region are about 10 times smaller than the total signal at 680 nm at all times. Other data are presented as transient spectra at certain time delays [$\Delta OD(\lambda,t)$], which are calculated from the kinetic spectra using eq 1 to obtain spectra with the precision gain from analysis of multiple datasets.

To avoid ambiguity, we define the usage of the terms component, lifetime, rate constant, and time constant in this paper in the following way: Each element of the summation defined in eq 1 above is referred to as a component. The terms "lifetime" and "time constant" are used interchangeably to refer to the exponential decay times τ_i defined in eq 1 for each component, and therefore are essentially experimentally observed parameters (assuming multiexponential kinetics). In contrast, the term "(intrinsic) rate constant" is used to refer to the rate constant of a specific electron or energy transfer reaction between two states. In general, and indeed for the system discussed here, these intrinsic rate constants can only be obtained from the experimentally observed time constants by the solution of a kinetic model, and the resulting intrinsic rate constants are therefore model-dependent. Under favorable circumstances, however, some conclusions about certain kinetic components can be drawn without going through the process of complete modeling. This is true for the processes occurring with 20−30 ps lifetimes in the photosystem II reaction centers, as will be shown in this paper.

We first of all consider transient absorption data collected in the region of the Q_y absorption band of the PS II reaction center. Figure 2 shows the time dependence of ΔOD data at 665 nm when the sample has been excited with a C680 selective pulse (trace (a)) versus excitation with a pulse photoselecting C670 chlorins (trace (b)). Global analyses of such data with the multichannel detector resolved the same number of components, with identical lifetimes within error, to those previously reported using single-wavelength detection (Durrant et al., 1992a,b, 1993; Hastings et al., 1992). As previously, components with lifetimes of 100 fs, 400−900 fs, 3−4 ps, and 20−30 ps and a nondecaying component were resolved from data obtained using either excitation wavelength [for details, see Durrant et al. (1993)]. Moreover, kinetic spectra obtained with the multichannel detector were similar to, but more detailed than, those reported previously with the single-wavelength detection. For example, as reported previously (Durrant et al., 1992b), the 100 fs

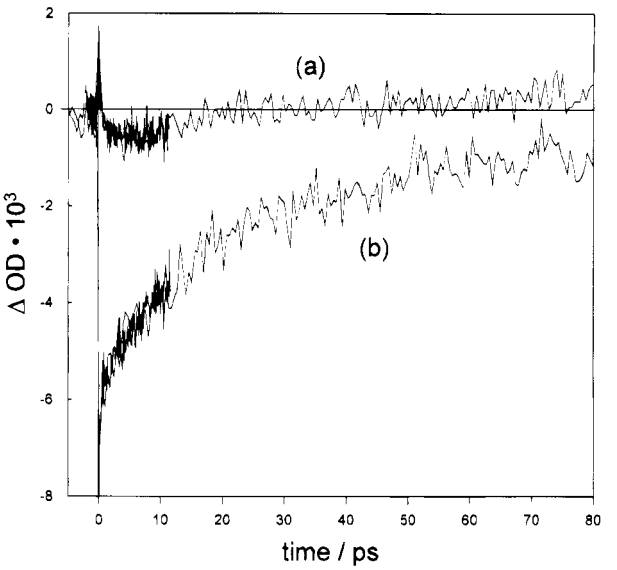

FIGURE 2: Transient absorption kinetics at 665 nm detection wavelength. Trace (a) using excitation with a pulse photoselecting C680 pigments, trace (b) using C670 photoselective excitation. This figure demonstrates the presence of a small but clearly resolvable amplitude of the 21 ps component after C680 photoselective excitation in comparison with the much larger amplitude of the 27 ps component under excitation conditions which generate slow energy transfer. The wavelength dependence of the amplitudes of these components is shown in Figure 3.

component distinguishes itself from other components by the complete inversion of the kinetic spectrum when C670 rather than C680 is photoselectively excited.

The greater spectral detail obtainable with the multichannel data allowed the resolution of previously unreported differences in the spectra of the 20−30 ps component depending upon excitation wavelength. It is this observation which is addressed in detail in this paper. These spectral differences are also associated with differences in the measured time constant. This difference in time constant was previously observed with our single-channel detector (Durrant et al., 1993).

Figure 3 shows spectra of the amplitude of the 20−30 ps components (kinetic spectra) taken using the multichannel detector and photoselective excitation of either C680 (solid lines) or C670 (dashed lines) chlorins. All spectra are the average of spectra obtained from 3−5 diffeent samples. Spectra were obtained using either the parallel (Figure 3a) or the magic-angle (Figure 3b) configuration of the pump and probe beam polarizations. The time constants for these components were determined to be, for the parallel configuration, 21 ± 1 ps (C680 excitation) and 27 ± 2 ps (C670 excitation). For the magic-angle configuration, the time constants obtained for the two excitation wavelengths were not obtained with such precision, and were indistinguishable (23 ± 3 ps). The parallel configuration increases the relative amplitude of pigments which are directly excited. In contrast, the magic-angle data are independent of pigment orientation. It is clear from this figure that not only the observed time constants but also the kinetic spectra of the 20−30 ps components can depend upon excitation wavelength. The difference in amplitude is also apparent in Figure 2.

Figure 4 shows transient spectra in the region of the pheophytin Q_x band centered at 545 nm following photoselective excitation of the C680 (Figure 4a) and C670 (Figure

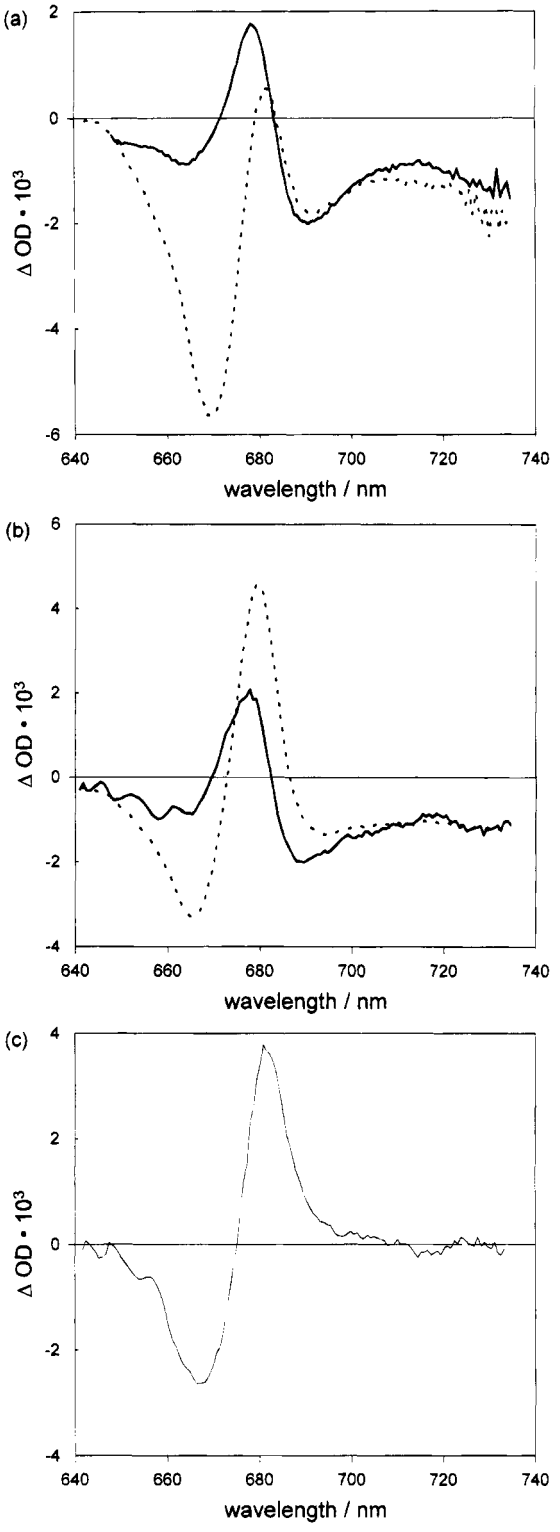

FIGURE 3: Spectra of the amplitudes (kinetic spectra) of 20−30 ps components associated with radical pair formation using parallel (a) or magic-angle (b) excitation. The solid lines are the spectra obtained using photoselective excitation of C680 chlorins, the dashed lines those obtained using photoselective excitation of C670 chlorins. The time constants of these components are 21 ± 1 ps (C680 excitation) and 27 ± 2 ps (C670 excitation) for parallel polarization; for the magic-angle configuration, the time constants were not obtained with such precision and were indistinguishable (23 ± 3 ps). The differences between the kinetic spectra for the two excitation wavelengths are due to slow energy transfer processes which are only observed using photoselective excitation of the C670 chlorin pool (see text). This difference is illustrated in Figure 3c, which shows the difference between the two spectra shown in Figure 3b.

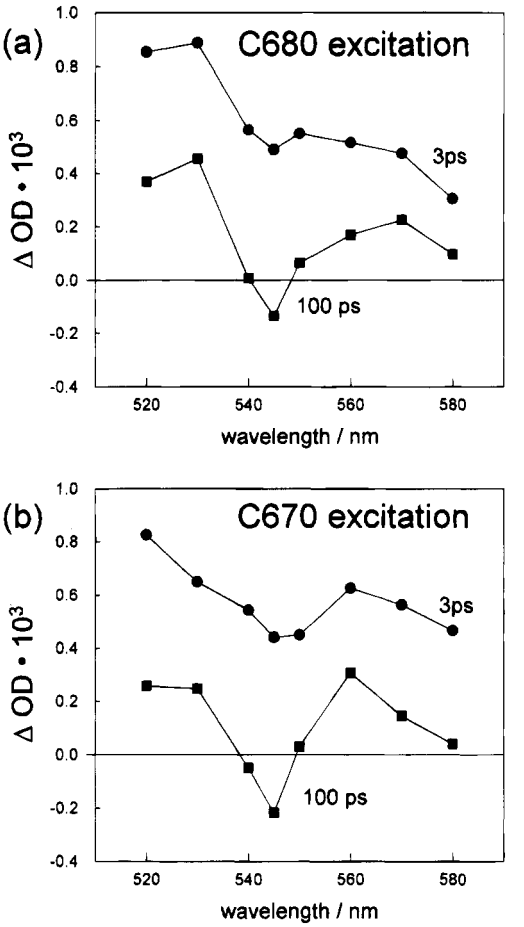

FIGURE 4: Transient absorption difference spectra at time delays of 3 and 100 ps observed using parallel photoselective excitation of (a) the C680 chlorins and (b) the C670 chlorins. These spectra correspond approximately to the spectra before and after the action of (a) a 21 ps and (b) a 27 ps component on the original transient spectrum. This figure demonstrates that the 21 and 27 ps components result in the same increase in the amplitude of pheophytin Q_x bleach.

4b) chlorin pools. The transient spectra shown are at time delays of 3 and 100 ps, and correspond approximately to the spectra before and after the action of the 21 ps (Figure 4a) and 27 ps (Figure 4b) components. Analyses of data collected in this spectral region were performed as described in Hastings et al. (1992). Time constants of 21 ± 2 and 27 ± 3 ps were recovered, the same values as those observed in the Q_y bands. The spectra shown in Figure 4a are similar to those previously published. The transient spectra at 100 ps are assigned to the radical pair state $P680^+Ph^-$ (Hastings et al., 1992; Durrant et al., 1993). This figure demonstrates that a lower limit of 60% of the final pheophytin Q_x bleach is produced by both the 21 ps and 27 ps components following excitation of either the C670 or the C680 chlorin pool. The small trough at 545 nm observed in the 3 ps spectra appears within 200 fs (data not shown), and is therefore assigned to excited singlet states of pheophytin formed by direct excitation or by energy transfer faster than 200 fs.

DISCUSSION

In discussing our results, we assume that the kinetics in PS II reaction centers can be appropriately described by a kinetic model consisting of a set of coupled differential equations, the solutions to which are eigenvalues of the

system corresponding to the observable kinetic components. The second assumption we make is that the excitation wavelength dependent change in the experimentally determined time constant/kinetic spectrum associated with charge separation (27 or 21 ps) is due to the addition of one process/state to the overall kinetics/kinetic spectra (see below). Inhomogeneity in this reaction center is much smaller than the separation of the two excitation pulses (Jankoviak et al., 1989), and inhomogeneous effects are therefore not included in our analysis.

The results shown in this paper demonstrate that there are experimentally determined kinetic components in PS II reaction centers which have time constants of either 21 ps or 27 ps depending on which of two chlorin pools (C670 or C680) is initially excited. Moreover, the spectra of these components clearly differ in the 670−680 nm region while being essentially identical at all other investigated wavelengths (see Figures 3 and 4). These two observations demonstrate that the two components contain contributions from a different mixture of states. Therefore, complete redistribution/equilibration of the excitation energy has not occurred prior to this 20−30 ps time scale. Although it has previously been demonstrated that the majority of chlorins in PS II reaction centers equilibrate on a subpicosecond time scale (Durrant et al., 1992b), our observations confirm that some slow energy transfer does occur. This is in agreement with a number of previous studies (Tang et al., 1990; Wasielewski et al., 1989b; Roelofs et al., 1991; Schelvis et al., 1993; Hastings et al., 1992).

Detailed consideration of the dependence of the spectra of the 20−30 ps components upon excitation wavelength supports the contribution of a slow energy transfer process to the data. Figure 3c shows the difference between the spectra of these components obtained using C670 and C680 magic-angle excitation (the difference between the kinetic spectra shown in Figure 3b). This spectrum shows the sigmoidal shape typical of a different distribution of energy between excited states, with the position of the maxima/minima specifically indicating transfer from 670 nm absorbing to 680 nm absorbing pigments.

Thus, the presence of slow energy transfer processes within the PS II reaction center is confirmed not only by the change in lifetime of the 20−30 ps components resolved for parallel polarization as a function of excitation wavelength (Figure 3a) but also by the change in shape of the kinetic spectra observed for magic-angle polarization (Figure 3b).

The important question is whether or not the observed time constant of formation of $P680^+Ph^-$ is limited by slow energy transfer. There are two observations that can be combined to show that both radical pair formation not limited by slow energy transfer and radical pair formation slowed by an energy transfer bottleneck coexist in PS II reaction centers. The dominant "process" depends upon excitation wavelength. Indeed, *only* the following two observations need to be combined to come to this conclusion, independent of the specific kinetic model chosen.

(1) The first observation is that the absorption of the C680 pool is dominated by P680. The absorption difference spectrum immediately after excitation ($t = 0 \pm 20$ fs) indicates that 694 nm excitation only excites molecules with Q_y absorption maxima near 680 nm (Durrant et al., 1992b). All previous attempts to deconvolute the Q_y absorption band of PS II reaction centers have assigned at least 50% (and often 100%) of the oscillator strength of the pigments with maxima near 680 nm to P680 (Montoya et al., 1993; van der Vos et al., 1992; Kwa et al., 1992; Braun et al., 1990; Otte et al., 1992). Therefore, 694 nm excitation must excite P680 directly in at least 50% of excited reaction centers, in agreement with our own observations that the 694 nm pulses excite species with a relatively high average oscillator strength (Durrant et al., 1992b).

(2) The second observation is that following 694 nm excitation, the majority (at least 75 ± 15%) of P680 oxidation occurs with a 21 ps time constant (Durrant et al., 1993; also discussed below).

However, when P680 is not photoselectively excited, an energy transfer process slows the overall time constant for pheophytin reduction and P680 oxidation to 27 ps. Thus, previous observations of slow energy transfer in PS II reaction centers and 21 ps charge separation not limited by slow energy transfer are not mutually exclusive.

At this point, it should be emphasized that the observed 21 and 27 ps time constants for radical pair formation do not correspond directly to electron transfer rate constants, but are complex combinations of all the intrinsic rate constants for the associated energy and electron transfer reactions. The difference in the lifetime of the two observed components results from different relative contributions from these reactions when excitation pulses having different spectra are used. If the difference between the 21 and 27 ps components is essentially due to a large change in the amplitude of one process or increased contribution from one state, then the interpretation of these results is rather straightforward.

In the following discussion, we expand upon some of the issues raised above in more detail.

Assignment of the 21 and 27 ps Components. The Q_y region of the PS II reaction center spectrum is a particularly congested one, with at least eight chlorins contributing to the overall spectrum. This makes assignment of kinetics in this region a difficult task. Because of this, we have previously associated radical pair forming steps with certain kinetic components by examining spectral regions which are far less spectrally congested (Hastings et al., 1992; Durrant et al., 1993). In particular, we have previously (Hastings et al., 1992) shown that if C680 is photoselected a lower limit of 60 ± 20% of the pheophytin Q_x bleach found in the radical pair $P680^+Ph^-$ is produced by the 21 ps component. It was also shown that 75 ± 15% of a stimulated emission band at 730 nm was lost with a 21 ps lifetime (Durrant et al., 1993). These and other observations led to the conclusion that photoselective excitation of the C680 pigments in PS II reaction centers leads to radical pair formation occurring primarily with a time constant of 21 ps (Hastings et al., 1992; Durrant et al., 1993).

As mentioned above, one of the key spectral regions for the assignment of radical pair formation is the pheophytin Q_x absorption band at 545 nm. Figure 4 shows transient spectra obtained in this spectral region. Photoselective excitation of either the C670 or the C680 chlorin pools produces identical spectra at both 3 ps and 100 ps. It can be concluded from these spectra that both the 21 and 27 ps components produce 60% of the final pheophytin Q_x bleach which is ultimately found in the radical pair $P680^+Ph^-$. Other data (not shown) determined that when C670 is excited a 27 ps lifetime is also associated with the appearance of the pheophytin anion band at 460 nm and loss of a stimulated emission band at 730 nm. The wavelength dependence of

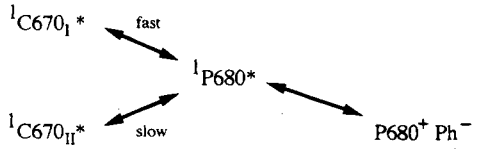

FIGURE 5: Illustrative diagram of isolated PS II reaction centers including energy transfer between two pools of C670 chlorins and P680, and charge separation. This diagram illustrates the presence of both fast and slow energy transfer processes in PS II, and is not a complete kinetic model of PS2's primary photochemistry (see text for details).

the 21 and 27 ps components was very similar over the 460, 545, and 730 nm spectral regions, and the association of the 27 ps component with radical pair formation is thereby confirmed. The spectra do, however, differ considerably in the vicinity of 670−680 nm, and it is this feature which we addressed above (Figure 3c).

Within the framework of a kinetic model based on processes with exponential rate constants, the fact that the lifetime of a component varies with excitation wavelength implies that it does not correspond to an eigenvalue of the model. Interpretation of the results in wavelength regions where the spectral shapes of the two components are identical, however, leads to the conclusion that both are associated with radical pair formation (see Figure 4). The spectral difference observed between C670 and C680 excitation can be attributed solely to the presence of a slow energy transfer process between C670 and C680 pigments whose amplitude depends upon which pigments are initially excited. This slow energy transfer occurs on the same time scale as excited states are converted into radical pairs. This leads to an increase appearent time constant associated with radical pair formation. The positive feature near 680 nm using C680 excitation in Figure 3 is consistent with the loss of the pheophytin absorption as Ph^- is formed. The negative regions red of 682 nm are dominated by the loss of stimulated emission from all excited chlorins in PS II and the appearance of radical pair absorption.

Fast and Slow Energy Transfer in PS II Reaction Centers. A diagram to illustrate this discussion is shown in Figure 5. This is not intended to be a kinetic model of PS II, as we have as yet been unable to assign all of the transient components observed in our studies of PS II reaction centers (see discussion below). Four states are included: excited singlet states of C680 chlorins, the radical pair $P680^+Ph^-$, and singlet excited states of two pools of accessory chlorins, $C670_I$ and $C670_{II}$. The C680 pigment pool is dominated by P680 [see discussion above and Durrant et al. (1992b)]. The presence of the $C670_I$ pool has been established previously by Durrant et al. (1992b). Excitation energy is rapidly transferred between C680 and the $C670_I$ chlorin pools, and excitation of either species results in approximately a 1:1 equilibration of excitation energy between these pigments within 100 fs, followed by radical pair formation associated with a time constant of 21 ps. Excitation energy transfer from the $C670_{II}$ to the C680 chlorin pools is relatively slow, which prevents equilibration of excitation energy between these pigments prior to radical pair formation. This results in a slower observed time constant for radical pair formation if $C670_{II}$ states are excited. Photoselective excitation of the C670 pigments will excite both the $C670_I$ and $C670_{II}$ chlorin pools, and will therefore result in a longer average time constant for radical pair formation than C680 selective excitation. The effect of reverse energy transfer from C680 to $C670_{II}$ chlorins is small as this energetically uphill step will be ~2.7 times slower, due to the energy separation of approximately 1 k_BT.

All the essential features in Figure 5, namely, slow and fast energy transfer and radical pair formation, are necessary to explain the experimental observations presented here and previously (Hastings et al., 1992; Durrant et al., 1992b, 1993). However, the diagram in Figure 5 is obviously insufficient to account for all the kinetics observed in PS II reaction centers as we already know that at least two additional components (lifetimes of approximately 600 fs and 3.5 ps) can be observed (e.g., Durrant et al., 1992a, 1993), and hence at least two additional states play a role in the overall kinetics. For example, while it has been shown that the 694 nm excitation pulses primarily excite P680 (Durrant et al., 1992b), there is also evidence for the presence of other chlorins/states within the PS II reaction center with Q_y peaks at 680 nm (Tang et al., 1990; Kwa et al., 1992; Kwa, 1993). It is possible that the 600 fs and 3.5 ps components may, at least in part, be due to energy transfer/exciton scattering between P680 and these other 680 nm absorbing chlorins [we have shown previously that these faster components are not primarily associated with charge separation (Durrant et al., 1993), in contrast to the conclusions of other groups (Wasielewski et al., 1989a; Jankoviak et al., 1989; Roelofs et al., 1991; Gatzen et al., 1992; Schelvis et al., 1994)].

The clear differences in the lifetime and spectrum of the 21 and 27 ps components show that none of the processes associated with the 100 fs, 600 fs, and 3.5 ps lifetimes leads to complete equilibration of the excitation energy when C670 is excited. Therefore, although these components are not discussed in detail in this paper, their existence does not invalidate the conclusions presented here. It does, however, make a quantitative analysis of the model illustrated in Figure 5 inappropriate. Moreover, such analyses are complicated by uncertainty in the number of chlorins associated with each pigment pool. The stoichiometry is difficult to determine due to the extensive spectral overlap in the Q_y bands. We have previously calculated that the intrinsic rate constant of energy transfer from the $C670_I$ pool to P680 is $(200 \pm 100\ \text{fs})^{-1}$, and estimate from our observations presented here that the energy transfer rate constant from the $C670_{II}$ pool to P680 is $(10-20\ \text{ps})^{-1}$ with the reverse rate constant being 2−3 times slower. However, uncertainty about the identity of the 600 fs and 3.5 ps components currently prevents an estimate of the intrinsic rate constant of charge separation.

The observation of slow energy transfer rate constants within a reaction center implies a surprisingly large physical separation or unusual orientation of the $C670_{II}$ chlorin(s) releative to the other reaction center pigments, as has been discussed in more detail elsewhere (Tang et al., 1990; Schelvis et al., 1994; van Gorkom & Schelvis, 1993). Histidines 118 of both the D1 and D2 polypeptides, both probably located near the exterior of the reaction center (Ruffle et al., 1992), are potential ligands to the $C670_{II}$ chlorin(s) (Schelvis et al., 1994).

This paper does not address the identity of 400−900 fs and 3−4 ps components observed in many studies of PS II reaction centers (Wasielewski et al., 1989a,b; Jankoviak et al., 1989; Roelofs et al., 1991; Gatzen et al., 1992; Durrant et al., 1992a, 1993; Schelvis et al., 1994), which will be discussed in detail elsewhere. In addition, we note that considerable caution should be taken in relating the results presented here with studies at lower temperatures. The

balance between forward and reverse energy transfer steps will be strongly affected by temperature, assuming that energy transfer equilibria follow Boltzmann behavior. A temperature-dependent study of energy transfer processes would be a useful contribution to disentangling the various energy transfer interactions which occur in PS II reaction centers, and it is likely that an increasing number of these will become apparent as the quality of data improves. However, if is clear that the kinetics which are observed will depend on the excitation spectrum used in a particular experiment, as creation of a different mixture of initially excited chromophores will change the proportion of energy transfer processes which contribute to a given experiment.

Conclusion. We have confirmed previous observations of slow energy transfer processes in PS II reaction centers. These slow energy transfer processes are, however, not observed when P680 is photoselectively excited. We therefore conclude on the basis of these and previous observations that, when P680 is photoselectively excited, the 21 ps time constant associated with radical pair formation is not limited by any slow energy transfer process.

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