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Femtosecond photodichroism studies of isolated photosystem II reaction centers

(photosynthesis/green plants/femtosecond spectroscopy)

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ABSTRACT **Photosynthetic conversion of light energy into chemical potential begins in reaction center protein complexes, where rapid charge separation occurs with nearly unit quantum efficiency. Primary charge separation was studied in isolated photosystem II reaction centers from spinach containing 6 chlorophyll *a*, 2 pheophytin *a* (Pheo), 1 cytochrome b_{559}, and 2 β-carotene molecules. Time-resolved pump-probe kinetic spectroscopy was carried out with 105-fs time resolution and with the pump laser polarized parallel, perpendicular, and at the magic angle (54.7°) relative to the polarized probe beam. The time evolution of the transient absorption changes due to the formation of the oxidized primary electron donor $P680^+$ and the reduced primary electron acceptor $Pheo^-$ were measured at 820 nm and 545 nm, respectively. In addition, kinetics were obtained at 680 nm, the wavelength ascribed to the Q_y transition of the primary electron donor P680 in the reaction center. At each measured probe wavelength the kinetics of the transient absorption changes can be fit to two major kinetic components. The relative amplitudes of these components are strongly dependent on the polarization of the pump beam relative to that of the probe. At the magic angle, where no photoselection occurs, the amplitude of the 3-ps component, which is indicative of the charge separation, dominates. When the primary electron acceptor Pheo is reduced prior to P680 excitation, the 3-ps component is eliminated.**

The detailed mechanism of primary charge separation in isolated reaction center (RC) complexes from photosynthetic organisms is an important topic of current interest (1–4). Femtosecond transient absorption spectroscopy has been used to probe a variety of issues regarding primary charge separation in the RCs of anoxygenic bacteria. It is of considerable interest to obtain the analogous data for photosystem II (PSII) from algae and higher plants because of the similarities between the bacterial and PSII RCs (5, 6). The first reported isolation of the PSII RC, the D1–D2–cytochrome b_{559} (cyt-b_{559}) protein complex, from higher plants by Nanba and Satoh (5) yielded a complex containing 4–5 chlorophyll *a* (Chl) molecules (6–8). Subsequent work (9, 10) demonstrated that the RC complex, as originally isolated, was quite unstable, and thus of limited use in many spectroscopic studies. Fortunately, simple modifications to the Nanba and Satoh procedure, such as substituting the detergent dodecyl β-maltoside (DM) for Triton X-100 (9) and adding an active enzymatic O_2-scrubbing system *in situ* (10), improve the stability of the PSII RC under prolonged illumination. The use of DM does not alter the spectroscopic properties of the RC as does Triton X-100 (11–13). Other changes in the isolation procedure have led to stable RCs that contain 6 Chl, 2 pheophytin *a* (Pheo), 1 cyt-b_{559}, and 2 β-carotene molecules per RC (14–17).

Carrying out detailed spectroscopic studies of PSII RCs is difficult because at room temperature the Q_y bands of all the chlorophylls and pheophytins within the RC overlap to give a composite band at 675 nm. Detailed analyses of the ground state spectra suggest that Chl molecules associated with the primary donor, P680, and at least one of the pheophytins absorb near 680 nm, while the remaining pigments absorb closer to 670 nm (11–13, 18–21). Danielius *et al.* (22) reported a 35-ns lifetime for $P680^+$–$Pheo^-$ in isolated PSII RCs, while the charge separation rate constant in PSII RCs was estimated, using fluorescence lifetime measurements, by Schatz *et al.* (23) for protein complexes containing approximately 80 Chl per P680. Wasielewski *et al.* (24) reported the direct measurement of the primary charge separation rate constant for the isolated PSII RC. These femtosecond transient absorption studies initially used PSII RCs stabilized either by using polyethylene glycol (suspended in 0.04% Triton X-100) (24) or by substituting DM for Triton in the isolation procedure (25). These materials gave charge separation $1/e$ times of 3.0 ± 0.6 ps at 277 K and 1.4 ± 0.2 ps at 15 K (26), based initially on the rise time of the transient absorption at 820 nm due to $P680^+$ (24) and later on both the 820-nm feature and the transient bleaching at 545 nm due to Pheo (25). In addition, Wasielewski *et al.* (26) found strong evidence at low temperature for the presence of an energy transfer process in the PSII RC that occurs with a 20- to 25-ps time constant. These results are in excellent agreement with the corresponding frequency domain data obtained from hole-burning spectroscopy at low temperatures (12, 13, 27). The 20- to 25-ps energy transfer process was also observed in fluorescence decay measurements using the original Nanba and Satoh preparation (28, 29). Recent fluorescence data from Roelofs *et al.* (30) at both 277 K and 77 K on RCs with 4–5 Chl molecules and from Gatzen *et al.* (31) at 277 K with RCs containing 6 Chl molecules revealed a 3-ps component attributed to charge separation and a 34-ps component assigned to energy transfer within the RC. More recently, Roelofs *et al.* (32) have observed a 3-ps fluorescence component in RCs with 6 Chl molecules at 277 K.

Using PSII RCs with 6 Chl molecules, Durrant *et al.* (33) and Hastings *et al.* (34) have obtained transient absorption data that have led them to propose that the formation time for $P680^+$–$Pheo^-$ is 21 ps. These conclusions are based primarily on observation of the appearance of the Pheo bleach at 545 nm. Within this data set they have also been able to identify several other kinetic components, including a 3.5-ps component. Some of these kinetic components are excitation wavelength dependent. More recently, Durrant *et al.* (35) have identified a 100-fs component that they attribute to energy transfer within the RC, and they have used stimulated

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Abbreviations: RC, reaction center; PSII, photosystem II; DM, dodecyl β-maltoside; Chl, chlorophyll *a*; Pheo, pheophytin *a*; cyt-b_{559}, cytochrome b_{559}.

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emission measurements to assess the various kinetic components associated with energy and electron transfer (36). McCauley *et al.* (37) used 50-fs 310-nm pulses to excite the PSII RC. Although only the bleaching at 672 nm was monitored, they observed a 13- (±4) ps recovery from the bleaching. Using picosecond pulses to study the absorption recovery in the Q_y region, Schelvis *et al.* initially (38) found no evidence for a 3-ps component, but they subsequently (39) observed this component, when the RCs were excited at the red edge of the Q_y absorption band.

The importance of the influence of pump and probe beam polarization on ultrafast measurements in PSII RCs was recently pointed out by Holzwarth *et al.* (40). The data presented in refs. 33–39 were obtained with the polarization of the pump laser beam parallel to that of the probe beam. Irradiation of the pigment array with a polarized laser beam preferentially excites those molecules oriented with their transition moment axes parallel to the polarization axis of the laser beam. To investigate these points further, we have carried out time-domain studies with 105-fs instrumental time resolution on both electron transport and energy transfer phenomena in stabilized DM PSII RCs at room temperature as a function of both pump and probe beam polarization relative to one another. Most important, we present measurements at the magic angle, 54.7°, at which no photoselection occurs (41).

MATERIALS AND METHODS

PSII RC complex was isolated from market spinach by a modification (9, 17, 42) of the procedure reported by Nanba and Satoh (5). The chromophore stoichiometry was checked (16), and this complex was found to contain 6 Chl molecules, 2 β-carotene molecules, and 1 cyt-b_{559} molecule per 2 Pheo molecules, similar to the stoichiometry published by others. These RCs are referred to as "native" in this paper and had a room temperature absorption peak at 674.6 nm at the time of the experiment and exhibited 87% of the activity of freshly isolated PSII RCs (43). Photodegradation of the RCs after prolonged exposure to light results in a 671-nm absorption maximum with <15% activity; therefore we avoided it. The PSII RCs were reduced by using sodium dithionite and methyl viologen in a manner reported earlier (24).

Transient absorption data were obtained by using a femtosecond dye laser system similar to that described previously (44). The 180-fs pulses of 585-nm light from the dye laser were frequency chirped in a 30-cm length of single-mode, polarization-preserving optical fiber, amplified at a 1-kHz repetition rate, and recompressed by using a prism pair to yield 75-fs pulses at 585 nm. Typically, 1-μJ pulses in a 0.5-mm spot were used to excite the samples in a nearly collinear pump-probe geometry, while intensity dependence experiments used pulse energies from 0.3 to 8 μJ. The sample had an absorbance of 1.0 at 675 nm and 0.11 at 585 nm in a 1-cm-pathlength cuvette that was rapidly stirred. Thus, about 15% of the RCs were excited. Kinetic parameters were obtained by iterative reconvolution using the Levenberg–Marquardt algorithm. Spectra at a given time were obtained by scanning the monochromator. The instrumental time response was 105 fs as determined by cross-correlation of the excitation and probe beams. All measurements were performed at 295 K.

RESULTS

Fig. 1 shows the transient absorption spectra obtained 10 ps after a 75-fs 585-nm laser flash for both native RCs and RCs in which the Pheo acceptor is prereduced. These data were obtained with the pump beam linearly polarized at 54.7° relative to the direction of the linearly polarized probe beam.

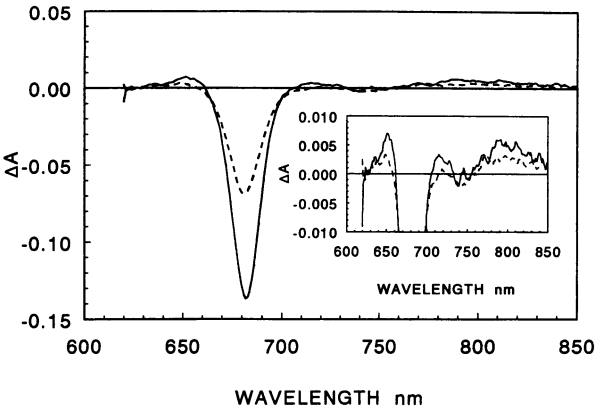

FIG. 1. Transient absorption spectra of PSII RCs obtained 10 ps after a 75-fs 585-nm laser flash. ——, Native RCs; ----, prereduced RCs. (*Inset*) Vertical magnification of the spectra. Spectra are determined with the pump beam polarized at 54.7° relative to the polarization of the probe beam.

The *Inset* to Fig. 1 shows that the transient absorption is larger throughout the 700- to 850-nm region in native samples. Prereduction of Pheo eliminates absorption changes that are due to the formation of $P680^+$–$Pheo^-$. Thus, the transient absorption spectrum of the prereduced sample reflects only contributions from the excited singlet states of the pigments. Excitation of about 15% of the RCs results in $[^1{}^*P680] = 3 \times 10^{-7}$ M because the concentration of RCs is 2×10^{-6} M. For both $P680^+$ (45) and $Pheo^-$ (46) $\Delta\varepsilon = 10^4\ M^{-1}\cdot cm^{-1}$, thus the total $\Delta\varepsilon$ for $P680^+$–$Pheo^-$ is $2 \times 10^4\ M^{-1}\cdot cm^{-1}$. The data in Fig. 1 show that ΔA at 820 nm is 0.005, so $[P680^+\text{–}Pheo^-] = 2.5 \times 10^{-7}$ M. Thus, 83% of the excited RCs result in formation of the $P680^+$–$Pheo^-$ radical pair. This is consistent with the measurement of 87% RC activity quoted above, and it implies that the active RCs undergo radical ion pair formation with nearly unit quantum yield.

Fig. 2 displays the transient absorption kinetics observed at 680 nm after the 75-fs 585-nm laser flash at three different orientations of the probe beam polarization relative to that of the pump beam. A close examination of Fig. 2 *Inset* shows that the kinetics are dominated by two processes, a fast initial recovery from the bleaching and a slower relaxation to the steady-state absorption change. The relative amplitudes of these changes depend on the relative polarizations of the pump and probe beams (Fig. 2 *Inset*). The slower relaxation process dominates when the pump beam is parallel to the

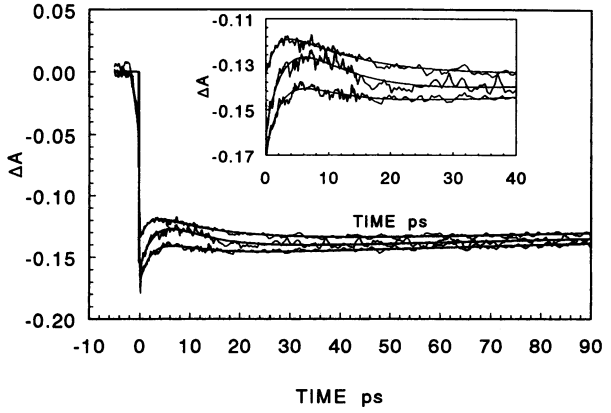

FIG. 2. Transient absorption changes of native PSII RCs at 680 nm after a 75-fs 585-nm laser flash for three orientations of the pump beam polarization relative to the orientation of the probe beam: top curve, parallel; middle curve, magic angle (54.7°); and bottom curve, perpendicular. (*Inset*) Magnification of the data in the main figure. Fits to the data are superimposed on the data.

Table 1. Transient absorption kinetic components for native PSII RCs at various orientations of pump beam polarization relative to probe beam polarization

Wavelength, nm	Magic angle		Parallel		Perpendicular	
	τ, ps	Amplitude	τ, ps	Amplitude	τ, ps	Amplitude
820	0.7	0.42	1.5	0.47	1.8	0.51
	4.0	0.58	6.2	0.53	3.6	0.49
680	2.6	0.75	1.6	0.48	2.3	0.78
	8.6	0.25	14.8	0.52	7.3	0.22
545	3.0	0.45	2.5	0.34	2.7	0.30
	180	0.55	194	0.66	156	0.70

Error limits on all components <20 ps are ±0.3 ps, while those on longer components are ±1 ps.

probe beam (top curve), while the short component dominates when the pump and probe are at the magic angle or at the perpendicular polarization. The time constants (τ) and relative amplitudes for these processes are given in Table 1 and will be discussed below.

Transient absorption changes at 820 nm, the maximum of the Chl^+ absorption (45), have been used to monitor the appearance of $P680^+$ in PSII. In Fig. 3 the polarization-dependent appearance kinetics for the formation of the 820-nm band in PSII RCs are shown. These kinetics are fit to two principal components. At the magic angle a 4.0-ps component dominates, but a 0.7-ps component of lesser amplitude also exists. The data for the amplitudes and time constants are given in Table 1. On the other hand, at the parallel polarization the two components are nearly equal in amplitude. The same can be said for the perpendicular polarization. While the slower, 4-ps, kinetic component observed in these data is comparable to the 3- to 3.5-ps component observed for the rise of the 820-nm band (24, 25), the enhanced signal-to-noise ratio and temporal resolution of the experiments described here reveal the presence of the shorter component. The poor quality of the fit at <1 ps is a consequence of kinetic complexity at these short times that cannot be adequately fit with two components, given the signal-to-noise ratio of the data.

In any attempt to assign transient absorption kinetics to the appearance of $P680^+$–$Pheo^-$, it is not sufficient to provide data for $P680^+$ alone; the $Pheo^-$ intermediate must also be identified. The Q_x absorption band of Pheo at 545 nm can be used to monitor the formation and decay of its excited and ionic states. However, the use of a spectroscopic change at 545 nm is not without difficulties. As is the case for 680 nm, more than one process is monitored at 545 nm. When Pheo is excited, the formation of ^{1}*Pheo results in bleaching of the 545-nm band (47). Further, when Pheo is reduced to $Pheo^-$ the 545-nm band also bleaches (46). The degree of bleaching is different, but the wavelength remains the same. Fig. 4 shows the transient absorption spectrum of the PSII RC at 5 ps after a 75-fs 585-nm laser flash for the native RC (solid curve) and for the prereduced RC (dashed curve). Note that the native RC is bleached more at 545 nm than is the prereduced material. Because the formation of $P680^+$–$Pheo^-$ is largely eliminated in the prereduced RC, the weaker bleaching in this RC is presumably due only to residual absorption changes involving formation of ^{1}*Pheo. Fig. 5 shows the kinetics of the absorption changes in native RCs for parallel (upper curve) and magic angle (lower curve) polarizations. Once again, the kinetics are complex. The kinetics can be fit to two dominant time constants, a fast 2.5- to 3.0-ps relaxation followed by a much slower 150- to 200-ps process at the magic angle and the parallel and perpendicular polarizations. The two components are nearly equal in amplitude at the magic angle, while the longer component strongly dominates at both parallel and perpendicular polarizations (Table 1). Fig. 6 shows a comparison of the 545-nm kinetics for native RCs (upper curve) with those of the prereduced sample (lower curve). In the prereduced sample the short component has almost disappeared and only the long component remains. These data suggest that the short component is associated with the formation of $P680^+$–$Pheo^-$, while the long component is not.

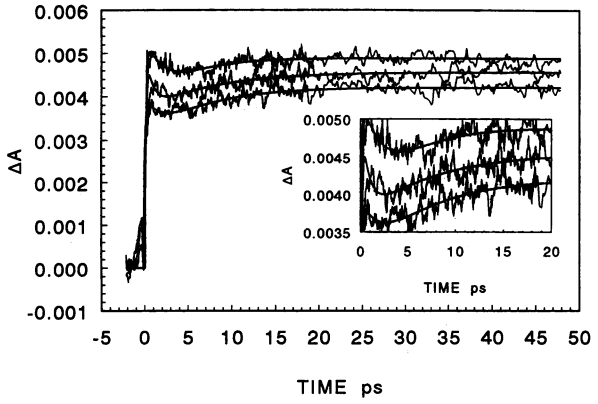

FIG. 3. Transient absorption changes of native PSII RCs at 820 nm after a 75-fs 585-nm laser flash for three orientations of the pump beam polarization relative to the orientation of the probe beam: top curve, parallel; middle curve, magic angle (54.7°); and bottom curve, perpendicular. (*Inset*) Magnification of the data in the main figure. Fits to the data are superimposed on the data.

DISCUSSION

Quantitative photodichroism measurements depend on exciting a single optical transition within a molecule with a polarized source. Excitation of the PSII RC, even with 690-nm light, can at best result in partial photoselection of P680 because the Pheo acceptor most likely absorbs near 680 nm (11–13). In

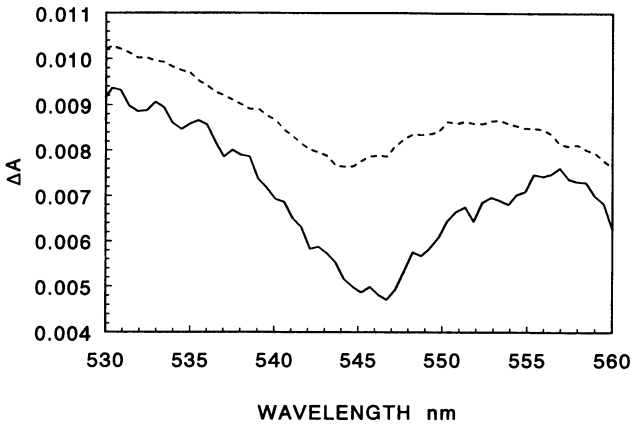

FIG. 4. Transient absorption spectra of PSII RCs obtained 5 ps after a 75-fs 585-nm laser flash. ——, Native RCs; ----, prereduced RCs. Spectra are determined with the pump beam polarized at 54.7° relative to the polarization of the probe beam.

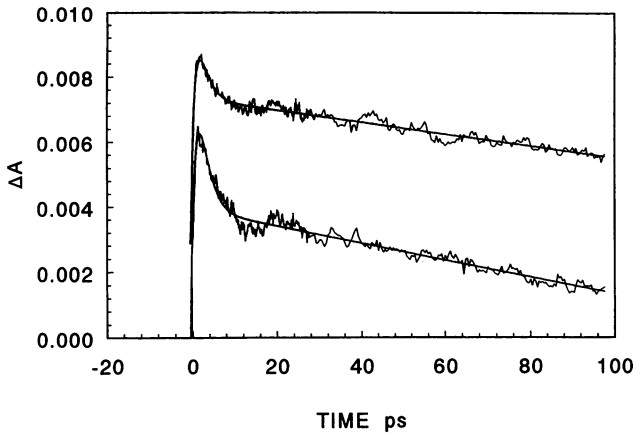

FIG. 5. Transient absorption changes of native PSII RCs at 545 nm after a 75-fs 585-nm laser flash for two orientations of the pump beam polarization relative to the orientation of the probe beam: lower curve, magic angle (54.7°); upper curve, parallel. Fits to the data are superimposed on the data.

addition, selective excitation of a subset of the pigments present in the PSII RC is complicated by the spectral width of femtosecond laser pulses due to uncertainty broadening. The spectral bandwidth of a transform-limited 75-fs laser pulse is 140 cm^{-1}, or about 6 nm at 585 nm and 7 nm at 690 nm. Energy transfer from other Chl or Pheo molecules in the reaction center to P680 will diminish the anisotropy observed in the transient absorption measurements. Nevertheless, the fraction of P680 that is directly excited by the laser flash may exhibit significant photoselection, even when the RCs are excited with 585 nm light. The degree of photoselection will of course be wavelength dependent.

The data presented in this paper demonstrate qualitatively that photoselection effects in PSII RCs are serious and can lead to significant differences in the observed amplitudes of the kinetic components at a variety of observation wavelengths. The sensitivity of the results to photoselection is not surprising in view of studies carried out on transient photodichroism in bacterial reaction centers (48). Changes in the transient absorption spectra of PSII RCs induced by photoselection with the polarized pump beam and measured by the polarized probe beam result in strong variations in the amplitudes of components in the multi-exponential kinetics observed in PSII RCs. This feature did not receive adequate attention in earlier work on the PSII RC, although work

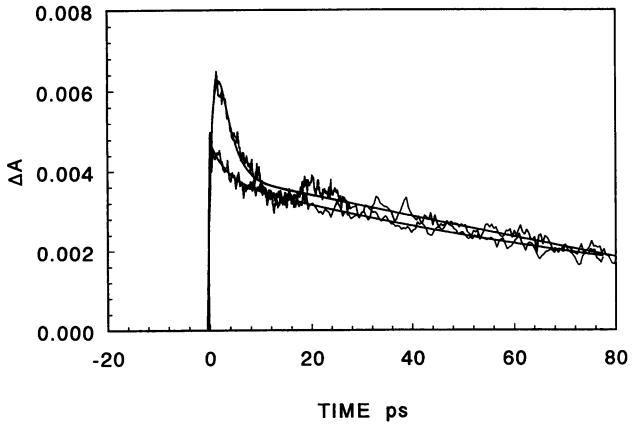

FIG. 6. Transient absorption changes of PSII RCs at 545 nm after a 75-fs 585-nm laser flash for native RCs (upper curve) and prereduced RCs (lower curve), with the orientation of the pump beam polarization set at 54.7° relative to that of the probe beam. Fits to the data are superimposed on the data.

described in our earlier report was carried out at the magic angle (24). In the work of Durrant *et al.* (33, 35, 36) and Hastings *et al.* (34) the pump and probe beams were polarized parallel to one another. Thus, significant photoselection effects may be present in their data, which may be a basis for differences in their interpretation of their data compared with our interpretation (refs. 24–26; this paper).

The data in Fig. 2 and Table 1 clearly show that the amplitudes of the two major kinetic components observed at 680 nm are sensitive to the relative polarization of the pump and probe beams. The 680-nm band strongly bleaches whenever any of the 8 Chl-like pigments in the PSII RC leaves its ground electronic state. It does not matter whether the product is an excited or ionic state. Thus, the amplitude of a kinetic component at a particular wavelength can be interpreted only within the context of the amplitudes of components observed elsewhere in the spectrum. Using parallel pump-probe polarization, we observe equal-amplitude 1.6- and 15-ps components at 680 nm. Hastings *et al.* (34) also observed a similar 18-ps component when they probed between 655 nm and 700 nm. Schelvis *et al.* (39) showed that a 25-ps component dominates the transient absorption kinetics in this wavelength region when the RCs are excited at wavelengths shorter than 680 nm for the parallel polarization. However, the direction of the absorption change is opposite to that which we and Hastings *et al.* (34) observe. This may be due to the differing excitation wavelengths used by each group. Table 1 shows that using a pump-probe polarization at the magic angle increases the amplitude of the short, 2.6-ps, component relative to that of the longer component.

Our simple hypothesis for the charge separation mechanism in PSII RCs is the reaction ^{1}*P680–Pheo $\rightarrow$ $P680^+$–$Pheo^-$. This mechanism is based on earlier work, and it recognizes the fact that there is no clear evidence in any preceding work for additional intermediates in this process. Thus, it is important for us to observe changes in the transient absorption kinetics due to the formation of both $P680^+$ and $Pheo^-$. In our initial paper we reported results on the formation of $P680^+$ (24), and in the following paper we reported work on both $P680^+$ and $Pheo^-$ (25). Durrant *et al.* (33, 35) have provided results on absorption changes at 545 nm that they attribute entirely to the production of $Pheo^-$. They performed these measurements with pump beam polarized parallel to the probe beam, which resulted in a dominant 21-ps kinetic component that they ascribed to the formation of $Pheo^-$. Our assignment of the 3-ps kinetic components at 820 nm and at 545 nm to the formation of $P680^+$ and $Pheo^-$, respectively, is based both on the dominance of the 3-ps component when no photoselection occurs and on the elimination of this same component when Pheo is reduced prior to excitation of the RC. Durrant *et al.* (33, 35) have not presented data showing the effects of Pheo prereduction on their kinetic components.

The spectroscopic results displayed in Figs. 1 and 4, obtained at the magic angle, once again confirm that our experiments are probing a state that differs from the locally excited states of the pigment array. When the PSII RC samples are prereduced, the spectral characteristics of the transient absorbance changes both in the near-infrared and at 545 nm differ significantly from those of the native RCs. The changes in the near-infrared can be ascribed to the fact that Chl and Pheo possess smaller molar extinction coefficients in this wavelength region than do their corresponding ions. This interpretation is supported by the data at 545 nm. At this wavelength any participation of a Pheo, either as an excited state or as an ion, will result in transient bleaching. In this case, at 5 ps, prereducing the sample significantly decreases the bleaching at 545 nm. The residual bleaching that is observed can be attributed either to a small amount of excited

state character on Pheo or to incomplete reduction of the active Pheo acceptor in the PSII RC.

The results presented in Figs. 3 and 5 show the effects of photoselection on the amplitudes of the kinetic components measured at 820 and 545 nm, respectively. At 820 nm both $P680^+$ and $Pheo^-$ absorb, while at 545 nm, the Pheo bleaching is attributable to formation of either ^{1}*Pheo or $Pheo^-$. Once again, the relative amplitudes of the two observed components in the kinetic traces depend on polarization. At 820 nm, the short decay component varies from 0.7 to 1.8 ps, and it may reflect a different process than does the 1.6- to 2.6-ps component observed at 680 nm. This process may be energy transfer or vibrational cooling of the pigments by the surrounding protein bath. The longer, rising component at 820 nm occurs over a 3.6–6.2-ps range and most likely monitors the formation of $P680^+$–$Pheo^-$. Note that the transient absorption change at 820 nm does not exhibit longer components out to 100 ps. The 4.0-ps component at 820 nm dominates at the magic angle. However, at the parallel and perpendicular polarizations the amplitudes of the two components are comparable. The lack of strong photoselection at 820 nm may indicate that the relative orientation of the transition dipoles for ^{1}*P680 and $P680^+$ may be close to the magic angle. The data obtained at 820 nm can be compared with those obtained at 545 nm. The short component for the bleaching at 545 nm occurs with a narrow range of 2.5–3.0 ps. Table 1 shows that the amplitude of the short component relative to that of the long component is strongly dependent on polarization. At the magic angle both components are of comparable magnitude. The long, 150- to 200-ps, component is consistent with that observed by Hastings *et al.* (34) when the RCs are excited at wavelengths shorter than 690 nm. However, at both parallel and perpendicular orientations the long components dominate. If we focus exclusively on data obtained at the magic angle, it is clear that there is a 3- to 4-ps process that is consistent with the data both at 820 and at 545 nm. We interpret this 3- to 4-ps process observed at 820 nm and 545 nm as the formation $P680^+$–$Pheo^-$. The longer components are most likely due to energy transfer processes within the RC (26, 31, 32, 38–40). The oscillatory behavior at early times in Fig. 5 is reproducible. However, at this time we do not wish to ascribe it to coherent processes within the RC, because it may simply be an additional kinetic complexity due to the many energy transfer pathways available within the PSII RC. This photodichroism study shows that data obtained at relative polarizations of the pump to probe beam other than the magic angle favor long kinetic components. The data in Fig. 6 show that prereduction of Pheo in the RC results in elimination of the 3-ps component, thus confirming the assignment of this component to formation of $P680^+$–$Pheo^-$. In addition, the fact that the long component remains in the kinetic response at 545 nm, even when Pheo is prereduced, suggests that the long component can be assigned to an energy transfer process within the PSII RC that is not connected to the P680 trap on this time scale.

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1. Deisenhofer, J. & Norris, J. R., eds. (1993) *The Photosynthetic Reaction Center* (Academic, New York).
2. Martin, J.-L., Breton, J., Hoff, A., Migus, A. & Antonetti, A. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 957–961.
3. Holzapfel, W., Finkele, U., Kaiser, W., Oesterhelt, D., Scheer, H., Stilz, H. U. & Zinth, W. (1989) *Chem. Phys. Lett.* **160**, 1–7.
4. Chan, C. K., DiMagno, T. J., Chen, L. X. Q., Norris, J. R. & Fleming, G. R. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11202–11206.
5. Nanba, O. & Satoh, K. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 109–112.
6. Gounaris, K., Chapman, D. J. & Barber, J. (1988) *FEBS Lett.* **234**, 374–378.
7. Barber, J., Chapman, D. J. & Telfer, A. (1987) *FEBS Lett.* **220**, 67–73.
8. Akabori, K., Tsukamoto, H., Tsukihara, J., Nagatsuka, T., Motokawa, O. & Toyoshima, Y. (1988) *Biochim. Biophys. Acta* **932**, 345–357.
9. Seibert, M., Picorel, R., Rubin, A. B. & Connolly, J. S. (1988) *Plant Physiol.* **87**, 303–306.
10. McTavish, H., Picorel, R. & Seibert, M. (1989) *Plant Physiol.* **89**, 453–456.
11. Tetenkin, V. L., Gulyaev, B. A., Seibert, M. & Rubin, A. B. (1989) *FEBS Lett.* **250**, 459–463.
12. Tang, D., Jankowiak, R., Seibert, M., Yocum, C. F. & Small, G. J. (1990) *J. Phys. Chem.* **94**, 6519–6522.
13. Tang, D., Jankowiak, R., Seibert, M. & Small, G. J. (1991) *Photosynthesis Res.* **27**, 19–29.
14. Gounaris, K., Chapman, D. J., Booth, P., Crystall, B., Giorgi, L. B., Klug, D. R., Porter, G. & Barber, J. (1990) *FEBS Lett.* **265**, 88–94.
15. Kobayashi, M., Maeda, H., Watanabe, T., Nakane, H. & Satoh, K. (1990) *FEBS Lett.* **260**, 138–140.
16. Montoya, G., Yruela, I. & Picorel, R. (1991) *FEBS Lett.* **283**, 255–258.
17. Chumanov, G., Picorel, R., Toon, S., Seibert, M. & Cotton, T. M. (1993) *Photochem. Photobiol.* **58**, 757–760.
18. Telfer, A. & Barber, J. (1989) *FEBS Lett.* **246**, 223–228.
19. Braun, P., Greenberg, B. M. & Scherz, A. (1990) *Biochemistry* **29**, 10376–10387.
20. Van Kan, P. J. M., Otte, S. C. M., Kleinherenbrink, F. A. M., Nieveen, M. C., Aartsma, T. J. & van Gorkom, H. J. (1990) *Biochim. Biophys. Acta* **1020**, 146–152.
21. Van der Vos, R., van Leeuwen, P. J., Braun, P. & Hoff, A. J. (1992) *Biochim. Biophys. Acta* **1140**, 184–198.
22. Danielius, R. V., Satoh, K., van Kan, P. J. M., Plijter, J. J., Nuijs, A. M. & van Gorkom, H. J. (1987) *FEBS Lett.* **213**, 241–244.
23. Schatz, G. H., Brock, H. & Holzwarth, A. R. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 8414–8418.
24. Wasielewski, M. R., Johnson, D. G., Seibert, M. & Govindjee (1989) *Proc. Natl. Acad. Sci. USA* **86**, 524–528.
25. Seibert, M., Toon, S., Govindjee, O'Neil, M. & Wasielewski, M. R. (1992) in *Research in Photosynthesis*, ed. Murata, N. (Kluwer, Dordrecht, The Netherlands) Vol. 2, pp. 41–44.
26. Wasielewski, M. R., Johnson, D. G., Govindjee, Preston, C. & Seibert, M. (1989) *Photosynthesis Res.* **22**, 89–99.
27. Jankowiak, R., Tang, D., Small, G. J. & Seibert, M. (1989) *J. Phys. Chem.* **93**, 1648–1654.
28. Mimuro, M., Yamazaki, I., Itoh, S., Tamai, N. & Satoh, K. (1988) *Biochim. Biophys. Acta* **933**, 478–486.
29. Govindjee, van de Ven, M., Preston, C., Seibert, M. & Gratton, E. (1990) *Biochim. Biophys. Acta* **1015**, 173–179.
30. Roelofs, T. A., Gilbert, M., Shuvalov, V. A. & Holzwarth, A. R. (1991) *Biochim. Biophys. Acta* **1060**, 237–244.
31. Gatzen, G., Griebenow, K., Muller, M. G. & Holzwarth, A. R. (1992) in *Research in Photosynthesis*, ed. Murata, N. (Kluwer, Dordrecht, The Netherlands), Vol. 2, pp. 69–72.
32. Roelofs, T. A., Kwa, S. L. S., van Grondelle, R., Dekker, J. & Holzwarth, A. R. (1993) *Biochim. Biophys. Acta* **1143**, 147–157.
33. Durrant, J. R., Hastings, G., Hong, Q., Barber, J., Porter, G. & Klug, D. R. (1992) *Chem. Phys. Lett.* **188**, 54–60.
34. Hastings, G., Durrant, J. R., Barber, J., Porter, G. & Klug, D. R. (1992) *Biochemistry* **31**, 7638–7647.
35. Durrant, J. R., Hastings, G., Joseph, D. M., Barber, J., Porter, G. & Klug, D. R. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 11632–11636.
36. Durrant, J. R., Hastings, G., Joseph, D. M., Barber, J., Porter, G. & Klug, D. R. (1993) *Biochemistry* **32**, 8259–8267.
37. McCauley, S. W., Baronavski, A. P., Rice, J. A., Ghirardi, M. L. & Mattoo, A. K. (1992) *Chem. Phys. Lett.* **198**, 437–442.
38. Schelvis, J. P. M., van Noort, P. I., Aartsma, T. J. & van Gorkom, H. J. (1992) in *Research in Photosynthesis*, ed. Murata, N. (Kluwer, Dordrecht, The Netherlands), Vol. 2, pp. 81–84.
39. Schelvis, J. P. M., van Noort, P. I., Aartsma, T. J. & van Gorkom, H. J. (1994) *Biochim. Biophys. Acta* **1184**, 242–250.
40. Holzwarth, A. R., Muller, M. G., Gatzen, G., Hucke, M. & Griebenow, K. (1994) *J. Luminesc.* **60/61**, 497–502.
41. Kliger, D. S., Lewis, J. W. & Randall, C. E. (1990) *Polarized Light in Optics and Spectroscopy* (Academic, New York), Chapt. 7.
42. Dunahay, T. G., Staehelin, L. A., Seibert, M., Ogilvie, P. D. & Berg, S. P. (1984) *Biochim. Biophys. Acta* **764**, 179–193.
43. Seibert, M. (1993) in *The Photosynthetic Reaction Center*, eds. Deisenhofer, J. & Norris, J. R. (Academic, New York), Vol. 1, pp. 319–356.
44. Wiederrecht, G. P., Watanabe, S. & Wasielewski, M. R. (1993) *Chem. Phys.* **176**, 601–614.
45. Davis, M. S., Forman, A. & Fajer, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4170–4174.
46. Fujita, I., Davis, M. S. & Fajer, J. (1978) *J. Am. Chem. Soc.* **100**, 6280–6282.
47. Overfield, R. E., Scherz, A., Kaufmann, K. J. & Wasielewski, M. R. (1983) *J. Am. Chem. Soc.* **105**, 4256–4260.
48. Kirmaier, C., Holten, D. & Parson, W. W. (1983) *Biochim. Biophys. Acta* **725**, 190–202.