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*Minireview*

# Structure and function in the isolated reaction center complex of Photosystem II: energy and charge transfer dynamics and mechanism

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**Abstract**

The dynamics of energy and charge transfer in the Photosystem II reaction center complex is an area of great interest today. These processes occur on a time scale ranging from femtoseconds to tens of picoseconds or longer. Steady-state and ultrafast spectroscopy techniques have provided a great deal of quantitative and qualitative data that have led to varied interpretations and phenomenological models. More recently, microscopic models that identify specific charge separated states have been introduced, and offer more insight into the charge transfer mechanism. The structure and energetics of PS II reaction centers are reviewed, emphasizing the effects on the dynamics of the initial charge transfer.

*Abbreviations:* 3PEPS – 3 pulse photon echo peak shift; Bchl – bacteriochlorophyll; BRC – bacterial reaction center from purple bacteria; Chl – chlorophyll *a*; FWHM – full width at half maximum; P – special pair in purple bacterial reaction centers; P680 – primary electron donor in Photosystem II, analogous to P; Pheo – pheophytin a; PS II – Photosystem II; RC – reaction center

## Introduction

Oxygenic photosynthesis in cyanobacteria, algae, and higher plants is a key chemical transformation supporting life on earth. The membrane bound pigment-protein complex known as Photosystem II (PS II) performs the initial reaction in oxygenic photosynthesis: the conversion of light energy into a redox pair capable of oxidizing water. Within the complex of proteins comprising PS II, the D1–D2-cytochrome *b*559 reaction center complex (RC) is responsible for the primary photoinitiated charge separation.

It is now well established by biochemical means that the RC complex of PS II contains 6 chlorophyll *a* (Chl) molecules, 1 or 2 $\beta$-carotene molecules, and 2 pheophytin *a* (Pheo) molecules (Kobayashi et al. 1990; Eijckelhoff and Dekker 1995). The physical arrangement, electronic structure and mechanistic roles for these pigments have been the subject of a multitude of biochemical, spectroscopic, and theoretical investigations (Diner and Babcock 1996; Dekker and van Grondelle 2000; Greenfield and Wasielewski 1996; van Grondelle et al. 1994). In particular, the spectroscopically identified P680 primary electron donor has been examined extensively. One can find elsewhere data and analyses that support a monomeric (Rutherford 1986; van Mieghem et al. 1991; van der Vos et al. 1992), dimeric (Kwa et al. 1994a; Noguchi et al. 1998; van Kan et al. 1990) or multimeric (Durrant et al. 1995b; Greenfield et al. 1999) electronic structure for P680. In general, P680 is thought to be analogous to the 'special pair' in purple bacteria, although it has been hypothesized that it is comprised of one 'special pair' Chl and one accessory Chl (van Gorkom 1995; van Gorkom and Schelvis 1993).

The recent advent of a low-resolution crystal structure for PS II isolated from *Synechococcus elongatus* (Zouni et al. 2001) has confirmed the pigment content

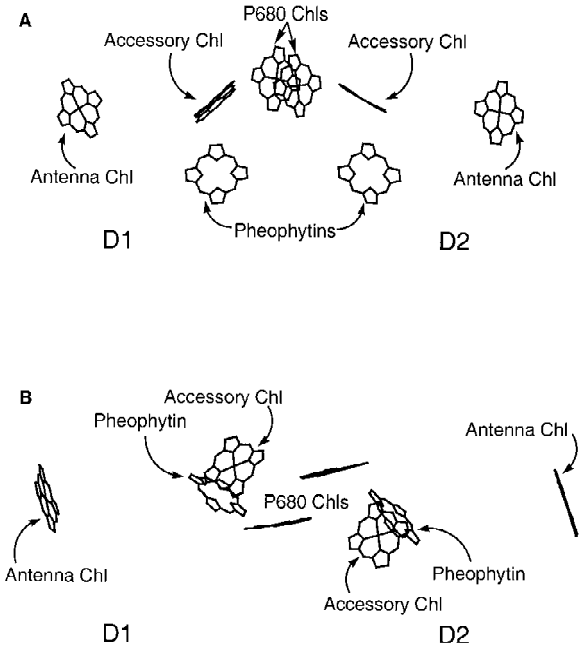


*Figure 1.* Crystal structure of the PS II reaction center from Zouni et al. (2001). The Chls are represented as porphyrins to illustrate their planar orientations and relative separations; the resolution is insufficient to determine the orientations of the Chl transition dipoles. (A) View along the membrane plane. (B) View onto membrane plane.

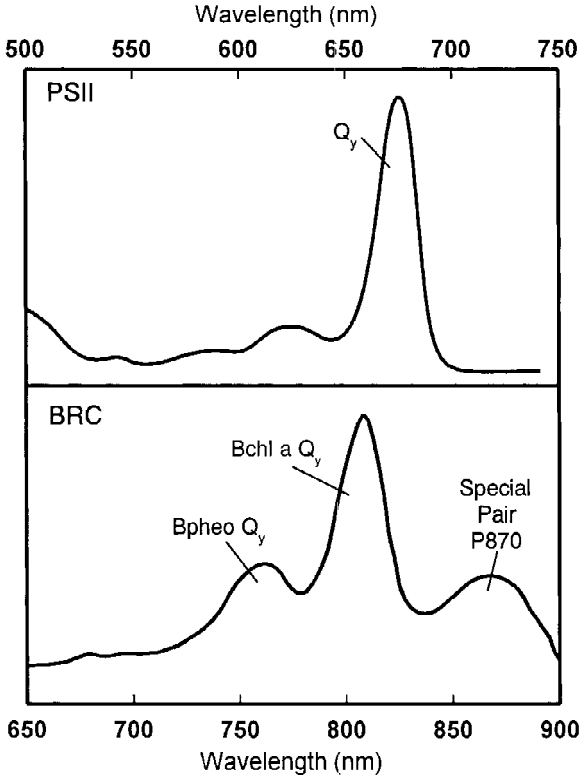


*Figure 2.* Absorption spectra of PS II (upper) and *R. sphaeroides* bacterial reaction center (lower), showing the overlapping $Q_y$ bands of PS II at 660 to 690 nm compared to the distinct special pair absorption in BRC at ca. 870 nm.

and provided new constraints, limiting the range of possible interpretations for the spectroscopic and kinetic data. However, this structure, shown in Figure 1, reveals the interpigment distances and the orientations of the pigment planes, but not the orientations of the transition dipoles. Without a high-resolution structure defining the orientation and positions of the pigments with angstrom resolution, the methods and data available provide only for indirect rather than direct elucidation of P680 and the mechanism of charge transfer. Thus, the mechanism of charge separation remains an open subject for investigation.

In this Minireview we will summarize the current state of knowledge of the spectroscopic, structural, and kinetic properties of the PS II reaction center, and explore the models proposed for the reaction center mechanism in the light of the available data.

## Steady-state spectroscopy of PS II

The interpretation of both steady state and time-resolved spectroscopic studies of PS II is complicated by the strong overlap of the spectra of all RC pigments as shown in Figure 2. At room temperature the $Q_y$ spectra of Chl and Pheo in solution have bandwidths comparable to the bandwidth of the $Q_y$ transition of the RC ($\Delta\lambda_{Chl,Pheo} \cong 18$ nm (ca. 400 $cm^{-1}$), $\Delta\lambda_{RC} \cong$ 25 nm (ca. 550 $cm^{-1}$)). Thus, it is difficult to separate the contributions of individual molecules or excitonic states and investigate their roles in energy and electron transfer in the RC of PS II.

The situation improves somewhat under low-temperature conditions (< 77 K). The widths of the individual bands decrease while the overall RC band width remains broad. Under these conditions the $Q_y$ bands of RC pigments are partially resolved (Konermann and Holzwarth 1996; van Kan et al. 1990). This allows one to distinguish the presence of short-wavelength Chl having maxima at $\lambda \sim 672$ nm and a long-wavelength form located at $\lambda$ = 680–683 nm.

Early assignments of the $Q_y$ band of Pheo were based on the isolated Pheo spectrum (Fragata et al. 1988), since the $Q_x$ band is resolved in the RC (544–545 nm) and can be compared with the $Q_x$ transition in isolated Pheo. Based on this comparison, the $Q_y$ band

is expected to appear at ca. 676 nm (Otte et al. 1992; van Kan et al. 1990). Further investigation has led to reports placing Pheo $Q_y$ peaks at different locations in the RC red band (Chang et al. 1994, 1995; Mimuro et al. 1995; Jankowiak et al. 1999). In general, it is now accepted that the active Pheo absorbs near 680 nm, but there is no consensus on the assignment of the inactive Pheo. In particular, the inactive Pheo $Q_y$ absorption has been reported at 668 nm (Jankowiak et al. 1999), 670 nm (Konermann and Holzwarth 1996; Konermann et al. 1997b; Dedic et al. 2000), 671 nm (Mimuro et al. 1995), 672 to 674 nm (Yruela et al. 1999), 676 nm (Germano et al. 2001) and near 680 nm (Shkuropatov et al. 1997).

Notwithstanding the active Pheo absorption near 680 nm, there is reason to believe (Kwa et al. 1992, 1994b; Satoh 1993; Seibert 1993; van Grondelle et al. 1994; Diner and Babcock 1996) that pump wavelengths on the red edge of the $Q_y$ band preferentially excite the Chl of the primary electron donor, P680. One study, however, presents evidence that the P680 transition is near 672 nm (Diner et al. 2001). The red edge absorption near 683 to 684 nm has been the subject of numerous studies. Several groups have attributed this band to the accessory $Chl_1$ (Konermann et al. 1997b; Germano et al. 2001; Diner et al. 2001). It has also been postulated that a loosely coupled Chl pigment may contribute to the red edge absorption in many preparations of the PS II RC complex. The loosely coupled Chl molecules associated with the core complex interact only weakly with the six central pigments, thus their influence on the energy levels is negligible. The loosely coupled Chl proposed to absorb at 683 to 684 nm has been identified as an energy transfer link between the antenna complex and the reaction center (Chang et al. 1994, 1995), and as one of the peripheral antenna Chls (Finzi et al. 1998, 1999; Jankowiak et al. 1999). Other groups have assigned peripheral antenna absorptions to the blue side, at 670 nm (Schelvis et al. 1994; Vacha et al. 1995; Germano et al. 2001).

## Pigment structure in PS II

The pigment structure in the PS II reaction center complex is illustrated in Figure 1. Within the 3.8–4.2 Å resolution of the crystal structure, the coordinates of the pigments of the reaction center have been tentatively assigned (Zouni et al. 2001). However, the orientations of the transition dipoles are not determined in the crystal structure. Thus the crystal structure provides a constraint, but not a determination of the functional structure for energy transfer and charge separation.

In the absence of a high-resolution crystal structure for PS II, additional insight into the reaction center may be obtained from the reaction center structures of purple bacteria. In fact, sequence homology and functional analogies between PS II and the bacterial reaction center (BRC) have led many investigators to use the BRC structure to model PS II (Deisenhofer et al. 1985; Durrant et al. 1995b; Svensson et al. 1996). In contrast to PS II, high-resolution crystal structures have been reported for several different reaction centers from purple bacteria (Deisenhofer et al. 1985, 1995; Yeates et al. 1988; Ermler et al. 1994; Nogi et al. 2000), allowing accurate determination of pigment locations and orientations.

Despite the structural and functional similarities, a critical difference between PS II and BRC is evident in their spectra, as illustrated in Figure 2. In all of the bacterial reaction centers, site energy shifts and strong excitonic coupling between the special pair of bacteriochlorophyll (Bchl) combine to lead to a low-lying absorption band, well isolated from the other pigment transitions. In *Rhodobacter sphaeroides* this absorption band is located at ca. 870 nm. In PS II, on the other hand, the spectrum is highly congested and there is no evidence for a 'special pair' of chlorophyll, with a trap state energetically isolated from the other pigments.

To first order, the special pair of BChl in the BRC can be considered as a dimer of identical chromophores. Assuming point-dipoles, the excitonic interaction energy is given by (see for example van Amerongen et al. 2000, Chapter 2):

$$V_{12} = \frac{\mathbf{D}_{\mathrm{oa}}^2 \chi}{R^3} \tag{1}$$

In this equation, $\mathbf{D}_{oa}$ is the dipole transition strength for the $Q_y$ transition of a chlorophyll monomer, $\mathbf{R}$ is the vector connecting the centers of the two chlorophyll monomers, having magnitude $R = |\mathbf{R}|$ and direction $\mathbf{r} = \mathbf{R}/|\mathbf{R}|$, and $\chi$ is an orientation factor:

$$\chi = (\mu_\mathbf{1} \cdot \mu_\mathbf{2}) - 3(\mathbf{r} \cdot \mu_\mathbf{1})(\mathbf{r} \cdot \mu_\mathbf{2}) \tag{2}$$

where $\mu_1$ and $\mu_2$ are the normalized transition dipole moment directions for the two chlorophylls. The parameters for *Blastochloris* (formerly *Rhodopsuedomonas*) *viridis* are $R = 7.6$ Å and $\chi = 1.26$ (Deisenhofer et al. 1985, 1995) and an *in vitro* dipole strength 51 $\mathbf{D}^2$

(BChl *b*) (Scherz and Rosenbach-Belkin 1988). The latter value corresponds to an *in vacuo* value of about 61 $\mathbf{D}^2$ for a refractive index $n = 1.34$ (see the correction factor in Shipman 1977). Thus, the interaction energy in *B. viridis* is $V_{12}$ = 740–880 $cm^{-1}$ with an excitonic splitting of $2V_{12}$ = 1480–1760 $cm^{-1}$. The analogous data for *R. sphaeroides* (Ermler et al. 1994), with a special pair of BChl *a*, are $R = 8$ Å, $\chi = 1.07$ and 33–39 $\mathbf{D}^2$ *in vitro* (Shipman 1977; Scherz and Rosenbach-Belkin 1988) (or about 40–48 $\mathbf{D}^2$ *in vacuo*). This yields an interaction energy $V_{12}$ = 350–500 $cm^{-1}$ and an excitonic splitting of $2V_{12}$ = 700–1000 $cm^{-1}$. The calculated values of $V_{12}$ are in good agreement with the experimentally measured splittings of *ca.* 1500–1700 $cm^{-1}$ in *B. viridis* and ca. 700–800 $cm^{-1}$ in *R. sphaeroides* (den Blanken and Hoff 1982; Knapp et al. 1985; Scherer and Fischer 1987; Reddy et al. 1993).

In PS II the pigments are Chl *a* with a monomer dipole strength of 17 $\mathbf{D}^2$ (*in vitro*) and 21–23 $\mathbf{D}^2$ (*in vacuo*) (Shipman 1977; Scherz and Rosenbach-Belkin 1988). For geometric parameters analogous to *B. viridis* or *Rb. sphaeroides* the interaction energy in PS II is predicted to be $V_{12}$ = 245–332 $cm^{-1}$ or $V_{12}$ = 179–242 $cm^{-1}$, respectively. These values imply excitonic splittings of 360 – 660 $cm^{-1}$ and are inconsistent with observations in PS II. To account for the decreased coupling between chlorophyll pigments, structural models for PS II based on the BRC have arbitrarily lengthened the separation between the pigments analogous to the bacterial special pair (Chang et al. 1994; Bosch et al. 1995; Durrant et al. 1995b; Svensson et al. 1996). The structure reported by Zouni et al. (2001), in Figure 1, supports this assumption and provides a center-to-center distance of ca. 10 Å. As a consequence of the reduction in 'special pair' coupling in PS II, the couplings between all of the core pigments are comparable in magnitude. This leads naturally to a multimer model for PS II (Durrant et al. 1995b).

Finally, it should also be noted that an alternative structural model for PS II, based on the 3.8 Å resolution crystal structure, was recently published (Vasil'ev et al. 2001). This model predicts a pigment separation of 8.3 Å, and an excitonic coupling of ca. 370 $cm^{-1}$, effectively introducing a special pair into PS II. This model is not consistent with the spectral data for PS II as described above.

## Ultrafast spectroscopic studies of PS II

Ultrafast time-resolved spectroscopies have been applied extensively to the investigation of photosynthetic systems in general, including PS II. Over the last decade many picosecond and femtosecond transient absorption and fluorescence studies have been published investigating the primary charge separation event in PS II. Results reported prior to 1996 are summarized in a review by Greenfield and Wasielewski (1996). The common feature in all of these studies is a wavelength dependent, multiexponential evolution of the spectrum and the excited state and photoproduct populations. Time constants range over four orders of magnitude, from ca. 100 fs to ca. 1 ns.

The recent transient absorption studies have expanded the range of pump and probe wavelengths and have revealed details of the temperature dependence. In emission experiments, a streak camera has been used to achieve an instrumental response function of 4.5 ps (Donovan et al. 1997), which is significantly narrower than the inherent resolution of single photon counting (ca. 30 ps; Gatzen et al. 1996; Konermann et al. 1997a). Even narrower sub-picosecond resolution can be obtained with fluorescence up-conversion, as demonstrated in a preliminary report (Kumazaki et al. 1995), but this technique is susceptible to complications due to multiply excited reaction centers from the necessary high photon flux. In addition, a photon echo experiment has been reported (Prokhorenko and Holzwarth 2000). Recent ultrafast studies not considered in the review of Greenfield and Wasielewski (1996) are summarized in Table 1.

The prevailing interpretation of the kinetic results seems to be that the electronic energy is equilibrated among the six central pigments of the reaction center on a sub-picosecond time scale. It is followed by charge separation with an intrinsic rate of (ca. 1 to 3 ps)$^{-1}$. Some observed components in the 10–50 ps range have been attributed to charge transfer following slow energy transfer from the weakly coupled antenna chlorophylls. Other long time constants are attributed to radical pair relaxation, due to either structural relaxation of the protein matrix or decay to different radical pair states.

## Site-directed mutants in PS II

A number of recent studies have compared mutant reaction centers with their analogous wild types, to

*Table 1.* Summary of ultrafast kinetic studies of PS II

| Reference | Method[a] | Excitation (nm) | Probe (nm) | $T$ (K) | Energy transfer[b] (ps) | Intrinsic charge transfer (ps) | Slow energy & charge transfer[c] (ps) | Radical pair relaxation (ps) |
|---|---|---|---|---|---|---|---|---|
| (Muller et al. 1996) | TA | 680/670 | 535–555<br>660–760 | 277 | $< 1$ | 2.4 | 8.9, 19.8 | 56 |
| (Merry et al. 1996) | TA | 665, 680, 694 | 660–760 | ca. 283 | 0.1, 0.6 | 3, 21 | | |
| (Groot et al. 1997) | TA | 685 | 655–700 | 20–240 | | 0.4–2.6 | 18–120 | |
| (Greenfield et al. 1997) | TA | 687 | 543, 558 | 278 | | $\leq 8$ | 50 | |
| (Greenfield et al. 1999) | TA | 661, 683 | 543.5, 558.5 | ca. 7 | $< 1$ | 5 | | |
| (Gatzen et al. 1996) | TRF | 620–689 | 660–690 | ca. 277 | | 3 | 6–30 | 50 |
| (Donovan et al. 1997) | TRF | 656–690 | 670–694 | 277 | | ca. 1 | 20 | |
| (Konermann et al. 1997) | TRF | 650 | 675–690 | 77 | $< 1$ | 1.4 | | 50 – 1 ns |
| (Kumazaki et al. 1995) | FU | 690 | 730 | | | 3, 21 | | |
| (Prokhorenko and) Holzwarth 2000) | 2PE | 674–688 | | 1.3 | ca. 100 fs | 1.5 | | 25 |

[a] TA – transient absorption; TRF – time-resolved fluorescence; FU – fluorescence upconversion; 2PE – 2 pulse photon echo.
[b] Energy equilibriation within the central core of RC pigments.
[c] Charge separation that is limited by the energy transfer rate from weakly coupled pigments.

gain further understanding into wild type structure and function. By altering specific protein residues, the binding characteristics of individual pigments can be altered. Specifically, spectroscopic and kinetic studies of isolated PS II reaction centers have employed mutants of *Synechocystis* sp. PCC 6803 and *Chlamydomonas reinhardtii*, with mutations at several different sites: D1-Gln130, which binds the active Pheo; D1-His198 and D2-His197, which bind P680; D1-His118 and D2-His117, which bind peripheral antenna Chls; and D2-Leu205, which is thought to interact with all D1 branch pigments. The main results of these studies are summarized here.

In a first study of the kinetics of mutant reaction centers, Giorgi et al. (1996) employed transient absorption to investigate *Synechosystis* D1-Gln130 mutants. They reported that charge separation in the mutant proceeds with the same time constant as the wild type, but with a varying yield of radical pairs. Subsequent studies by the same group on *Synechosystis* D1-Gln130 and D1-His198 mutants gave similar results: radical yields are affected by mutations near P680 and the active Pheo (Durrant et al. 1998; Merry et al. 1998). The authors concluded that the mutations effectively alter the relative energetics of the charge separated states, and that the radical pair yields are sensitive to fluctuations in the energetics (Merry et al. 1998). In time resolved studies of *C. reinhardtii,* D2-Leu205 mutants exhibited slower charge transfer kinetics than the wild type (Andronis et al. 1999). This experiment showed that the differing identities of the particular residue at D2-205 and the corresponding location in BRC do not account for the different charge separation dynamics between BRC and PS II.

Other time-resolved mutant studies have focused on D2-His117 and D1-His118 in *Synechocystis* (Vasil'ev and Bruce 2000) and *C. reinhardtii* (Johnston et al. 2000). Both groups found that the mutations affect the kinetic component attributed to charge separation following slow energy transfer, providing further evidence that the weakly coupled peripheral antenna chlorophylls are involved in this process.

Spectroscopic studies of *Synechocystis* mutants have led to further understanding of the identities of wild-type pigment transitions. Stewart et al. (2000) identified transitions for the Pheos, P680, and $Chl_1$ (the accessory chl on D1) that are in keeping with the previously mentioned wild type data. Diner et al. (2001) have reported that D1-His198 and D2-His197 mutations affect the reduction potential of P680, and have identified the accessory chlorophyll, $Chl_1$, as giving rise to a red-absorbing transition (684 nm). They propose, based on these results, that $Chl_1$ is the primary electron donor at low temperatures.

## Mechanism and models of energy and charge transfer

The detailed mechanism of energy and charge transfer in PS II reaction centers has long been an issue of discussion. Definitive answers have been elusive, in part because of the extensive spectral overlap among the pigment absorptions and the lack of a crystal structure at atomic resolution as discussed above. In models of PS II dynamics based on time resolved experimental data, the kinetics have typically been fitted with sums of exponential decays, with each component assigned to energy or charge transfer. The approach of fitting data to multiple exponentials, while providing a convenient means of representing the data, has limited ability to reveal the details of the microscopic process. Many fitting parameters are obtained, but their physical interpretation is unclear and often speculative. Indeed, in a disordered system with divergent kinetics (such as PS II), one would expect a simple exponential decay model to be inadequate. To more accurately represent the disordered reaction center environment, several groups have performed data analyses by fitting with a distribution of rate constants (Govindjee et al. 1990; Konermann et al. 1997a; Johnston et al. 2000). This method provides a more robust fitting algorithm, and its intuitive interpretation is appealing. However, the same uncertainties associated with assigning the fitted time constants remain.

Recent experimental and theoretical efforts have begun to go beyond phenomenological models to elucidate the microscopic details of energy and charge transfer in PS II. In a first step toward this end, Durrant et al. (1995b) introduced the multimer model for energy transfer, which showed that the excitation energy in the reaction center could be delocalized over multiple pigments. This model is supported by experimental evidence that the initially excited state is delocalized (Greenfield et al. 1999), and that the first charge transfer step proceeds from a delocalized electronic state (Peterman et al. 1998). Spectroscopic investigations of reaction centers with modified pheophytin composition have also indicated the importance of excitonic delocalization, with the additional insight that excitonic interactions are more pronounced on the active branch than on the inactive branch (Germano et al. 2001).

Microscopic models of the charge transfer steps in PS II, to a first approximation, can be based on the analogous process in bacterial reaction centers (Kirmaier and Holten 1993). The main route of charge separation in bacterial systems begins with energy transfer to the special pair (P), which donates an electron to the accessory bacteriochlorophyll (Bchl *a*) to form $P^+$Bchl $a^-$. This state subsequently decays to $P^+H_A^-$ where $H_A$ is the bacteriopheophytin on the L branch. This charge separation is completed on a time scale of ca. 3 ps. In PS II, there is no special pair to trap the excitation energy, but the comparable model is given by:

$$\begin{aligned} P680^*\,Chl_1Pheo_1 &\rightarrow P680^+Chl_1{}^-Pheo_1 \\ &\rightarrow P680^+Chl_1Pheo_1{}^- \end{aligned} \qquad (3)$$

where P680 is the PS II analogue of P, $Chl_1$ is the accessory chlorophyll on D1, and $Pheo_1$ is the pheophytin on D1. The electronic excitation is indicated by (*). Experimental evidence has been presented for the existence of a chlorophyll anion state, such as the intermediate state in Equation (1) (Durrant et al. 1998). The relative free energy of this state has not been measured, but quantum chemical calculations indicate that it is nearly isoenergetic with the excited singlet state (Blomberg et al. 1998). The final charge separated state ($P680^+Pheo_1{}^-$) has been estimated to lie below the excited singlet state by 160–950 $cm^{-1}$, based on recombination fluorescence studies (Booth et al. 1990; Roelofs et al. 1991; Volk et al. 1993; Groot et al. 1994). Quantum calculations place this state at nearly the same energy as both the excited singlet state and the intermediate charge separated state (Blomberg et al. 1998). The low driving force for charge separation has been implicated as a possible cause for the relatively slow charge separation kinetics compared with that of bacterial reaction centers (Dekker and van Grondelle 2000).

Recently, we have developed a microscopic model that describes energy and charge transfer in room temperature PS II using the charge separation scheme similar to Equation (3) (J.J. Shiang, L. M. Yoder and R. J. Sension, submitted for publication). In this model, the excited singlet states and charge transfer states are coupled with a protein matrix, and the master equation is solved to determine the time dependence of charge transfer. The energetics of the excitonic states are based on the multimer model (Durrant et al. 1995b), and the charge transfer couplings are derived from *ab initio* calculations of bacterial reaction centers (Zhang and Friesner 1998). An advantage of this model over phenomenological curve fitting is that the input parameters are deduced from independent theoretical and experimental work, so their physical meaning is clear. The model accurately simulates the

experimentally observed emission kinetics and their wavelength dependence.

Other possible locations of the charges and the excitation energy must also be considered in a more complete model. Several minor energy and charge transfer pathways have been identified in BRC; they involve excitation and a cation localized on the accessory bacteriochlorophyll (van Brederode and van Grondelle 1999). Recent studies have demonstrated that such a channel may be important in PS II as well. Diner et al. (2001) propose a mechanism for charge separation based on their spectral study of PS II mutants. They do not include excitonic coupling in their analysis, thus the donor state is not delocalized, but attributed to a specific pigment, the accessory chlorophyll. At low temperature the mechanism is proposed to be:

$$\begin{aligned} \mathrm{P680\ Chl_1{}^*\ Pheo_1} &\rightarrow \mathrm{P680^+Chl_1{}^-Pheo_1} \\ &\rightarrow \mathrm{P680^+Chl_1Pheo_1{}^-} \end{aligned} \tag{4a}$$

$$\begin{aligned} \mathrm{P680\ Chl_1{}^*\ Pheo_1} &\rightarrow \mathrm{P680Chl_1{}^+Pheo_1{}^-} \\ &\rightarrow \mathrm{P680^+Chl_1Pheo_1{}^-} \end{aligned} \tag{4b}$$

Charge separation proceeds by two possible intermediate states, which likely have similar energetics. Diner et al. (2001) also note that at room temperature the traditional mechanism (Equation (3)) is also expected to occur, although the mechanism in Equations (4a) and (4b) is three times more likely. A similar model has been proposed by Prokhorenko and Holzwarth (2000) to explain their photon echo experimental results at low temperature. Their model accounts for both energy and charge transfer observed in the dephasing of the initially excited state, and in low temperature transient absorption experiments:

$$\begin{aligned} &\text{(RC core)}^{*\prime} \rightarrow \text{(RC core)}^* \rightarrow \mathrm{P680\ Chl_1{}^+Pheo_1{}^-} \\ &\quad \rightarrow \mathrm{P680^+Chl_1Pheo_1{}^-} \end{aligned} \tag{5}$$

In this scheme the pigments are excitonically coupled so that the initially excited state (RC core)$*'$ becomes delocalized over the reaction center (RC core)$^*$ in 100–500 fs. The intermediate charge transfer state, formed with an intrinsic rate constant of ca. 1.5 ps, is attributed uniquely to $\mathrm{Chl_1{}^+Pheo_1{}^-}$. These charge transfer mechanisms are illustrated in Figure 3.

From these studies, it appears that multiple charge transfer pathways are possible in PS II and detailed microscopic models should account for them. Models of fluorescence may not be able to distinguish between different intermediate radical pair states, since these states do not emit; thus our model as described above succeeds for fluorescence simulations with a single intermediate state. However, extension of such a model to simulate transient absorption may require inclusion of the $\mathrm{Chl_1{}^+Pheo_1{}^-}$ state.

## Static disorder

The spectroscopic line width for transitions to the singlet excited state in PS II has contributions from both homogeneous and inhomogeneous broadening. Homogeneous broadening results from the coupling of the electronic transition to intramolecular vibrations of the chromophore and the coupling to lattice vibrations (phonons) associated with the protein matrix. The degree of coupling depends on the thermal populations of the coupled vibrations, thus homogeneous broadening is highly temperature dependent. Inhomogeneous broadening results from static disorder in the electronic energy of the transition due to the specific random protein environment, and is generally assumed to be temperature independent. The total width of the transition (FWHM) is given by $\Gamma^2 = \Gamma_{\mathrm{inh}}{}^2 + \Gamma_{\mathrm{hom}}{}^2$ where $\Gamma_{\mathrm{inh}}$ and $\Gamma_{\mathrm{hom}}$ are the FWHM of the inhomogeneous and homogeneous components, respectively (van Amerongen et al. 2000, Chap. 5). Spectral investigations of PS II over a wide temperature range have shown that at room temperature the linewidth is dominated by homogeneous broadening (Cattaneo et al. 1995; Konermann and Holzwarth 1996). Values of $\Gamma_{\mathrm{hom}}$ at room temperature range from 180 $\mathrm{cm^{-1}}$ for pheophytin to 324 $\mathrm{cm^{-1}}$ for P680 (Cattaneo et al. 1995). Hole burning experiments have consistently shown that the inhomogeneous broadening is on the order of 100 $\mathrm{cm^{-1}}$ (Jankowiak et al. 1989; Tang et al. 1990, 1991; Chang et al. 1995).

Microscopic models of energy transfer in the reaction center require consideration of static disorder with varying levels of detail. The total static disorder is comprised of diagonal and off-diagonal disorder. Off-diagonal disorder accounts for variations in the couplings between electronic states. To date, models have neglected off-diagonal disorder. Diagonal disorder accounts for static shifts in the energies of the electronic states. Simple diagonal disorder has been included successfully in models of hole burning, transient absorption and emission experiments. Recently, however, with the advent of photon echo experiments, more detailed representations of static disorder have become necessary for modeling the data,

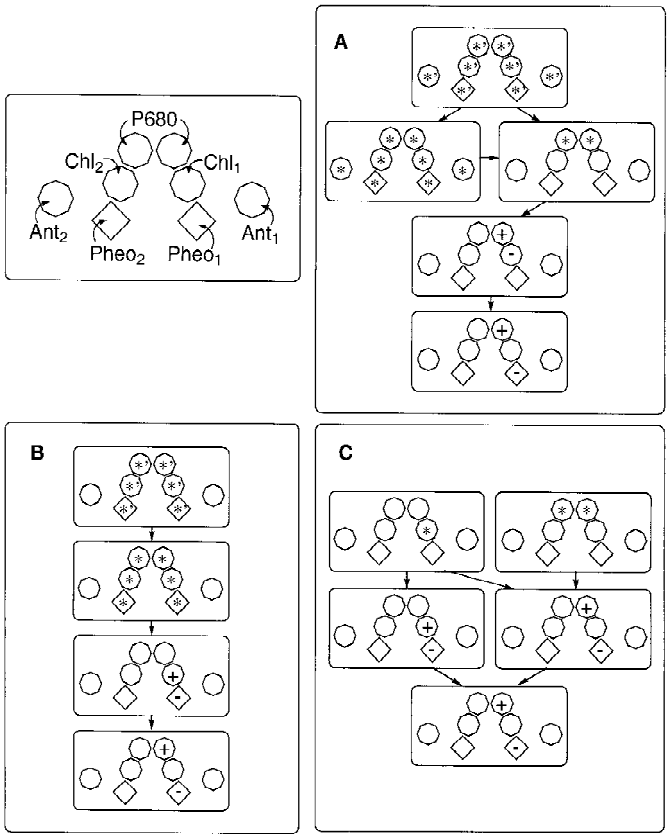


*Figure 3.* Models for charge transfer. *, initial electronic excitation; *, electronic excitation after energy transfer; + and –, cation and anion. (A) From Shiang, Yoder and Sension, submitted for publication; (B) from Prokhorenko and Holzwarth (2000); (C) from Diner et al. (2001).

including distinguishing between correlated and uncorrelated disorder (Yang and Fleming 2000; Agarwal et al. 2001), discussed in more detail below.

The simplest way to incorporate static disorder in a microscopic model of energy transfer is to assume uncorrelated Gaussian distributions (with FWHM $\Gamma_{inh}$) of site energies distributed around each of the electronic states. This approach is used in the 'multimer' model of energy transfer (Durrant et al. 1995b) and in our energy and charge transfer model (Shiang et al. submitted for publication). The intermolecular couplings in PS II are on the order of 20 to 100 $cm^{-1}$, so $\Gamma_{inh}$ on the order of 100 $cm^{-1}$ can potentially affect the energy transfer dynamics. Durrant et al. (1995b) found in their numerical simulations that the degree of exciton delocalization weakly depends on the width of the static distribution, for reasonable values of $\Gamma_{inh}$. For example, for values of $\Gamma_{inh} \leq 200$ $cm^{-1}$, the exciton remains delocalized between 3 to >4 pigments.

As more detailed experimental data become available, more sophisticated ways of accounting for static disorder have become necessary. Recent three pulse photon echo peak shift (3PEPS) studies of B800 in the bacterial light harvesting complex of *R. acidophila* have illustrated the distinction between correlated and uncorrelated disorder (Agarwal et al. 2001; Yang and Fleming 2000). (This technique has not been applied to PS II, but the similarities between bacterial systems and PS II point to the future importance of these ideas for the study of PS II.) In this model, a given reaction center has a degree of uncorrelated disorder, $\sigma$, between the individual pigments, and all the pigments of that reaction center have a common (correlated) static shift, $\Sigma$, relative to other reaction centers of the ensemble. Thus, the total width of the static distribution is $\Gamma_{inh}^2 = \Sigma^2 + \sigma^2$. The 3PEPS technique is able to distinguish correlated from uncorrelated disorder, with both components contributing significantly in B800 ($\Sigma = 50$ $cm^{-1}$; $\sigma = 90$ $cm^{-1}$) (Agarwal et al. 2001).

While experiments have revealed the static disorder of the singlet excited states from hole burning and temperature dependent spectral data, there are no comparable data for charge separated states. However, there is indirect evidence that points to static disorder in the radical pair states as a possible cause of the observed multiexponential kinetics in transient absorption and emission kinetics (Booth et al. 1991; Greenfield and Wasielewski 1996; Groot et al. 1994). Radical pair recombination studies of PS II (Groot et al. 1994) and BRC (Ogrodnik et al. 1994) have been interpreted through models that estimate the static disorder to be on the order of $\Gamma_{inh}$ = ca. 500–800 $cm^{-1}$. This estimated disorder is significantly larger than that for the singlet excited states, and it is roughly equal to the estimated $\Delta G$ in PS II, where $\Delta G$ is the free energy difference between the singlet excited state and the charge separated state, $P680^+Pheo^-$. The relatively large energetic width of the charge separated state may result from the corresponding increase in dipole moment, which may make the radical state more sensitive than the neutral excited state to the specific protein arrangement.

Recently, Barter et al. (2001) modeled the multiexponential kinetics of time-resolved fluorescence experiments using a three-step sequential mechanism that relies on the inclusion of significant static disorder. Previously, the same group showed that an analogous two-step model inadequately fit the data (Durrant et al. 1995a). Konermann et al. (1997a) also found that a similar two-step model with a static distribution gave poor agreement with their fluorescence data. In the successful 3-step phenomenological representation (Barter et al. 2001), electron transfer proceeds from an initial donor state to two sequential charge-separated states. The charge separated states have significant static disorder, which is necessary for the model to produce multiexponential kinetic behavior. While able to reproduce the experimentally observed kinetic trends, this model does not provide information on the microscopic identities of the states, nor does it take into account the kinetics of energy transfer between the reaction center pigments.

Because the couplings between electronic states, the variations in site energies and the energetic disorder are all of the same magnitude, a detailed model of the dynamics of PS II must account for all of the above, as in our model (Shiang et al., submitted for publication). We find that the predicted kinetics and radical pair yield depend sensitively on the inhomogeneous width. As the static disorder increases, the calculated radical pair yield decreases, and the kinetic profile becomes more divergent, giving the appearance of more exponential components, as shown in Figure 4. Clearly, any proposed mechanism or modeling scheme must account for the effects of static disorder.

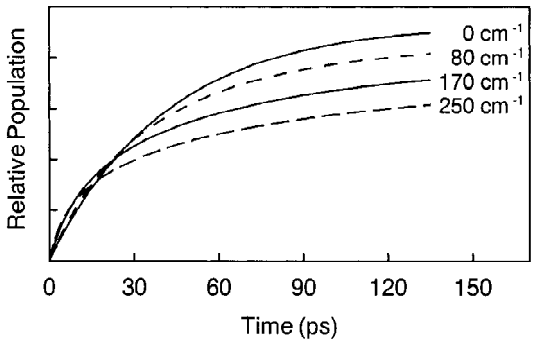


*Figure 4.* Calculated population kinetics of the $P680^+Pheo^-$ radical pair with static disorder from 0 to 250 $cm^{-1}$ (FWHM).

## Conclusions

Ultrafast spectroscopic measurements have identified the key processes of energy and charge transfer in PS II. After excitation of the reaction center, energy is equilibrated within a femtosecond to picosecond time scale. The excited state is likely to be excitonically delocalized over multiple pigments. Charge separation occurs on a picosecond time scale, and longer time components are evident due to the interplay between charge transfer and slower energy transfer from peripheral Chls and the reaction center, as well as interactions with the protein matrix. There is still no consensus, however, on the degree of excitonic delocalization, the specific assignments of the various observed components, or the details of the charge transfer mechanism.

To approach the problem from a theoretical perspective, a high resolution crystal structure is necessary. While there has been recent progress in this area, simulations still rely on the analogous bacterial reaction center structure. To model most accurately the dynamics in PS II, simulations should include multiple charge separated states, like those shown in Figure 3, and take proper account of static disorder in the system. To integrate fully the experimental and theoretical models, progress is needed in several areas: (1) a high-resolution crystal structure; (2) accurate calculations of the relative energies of all charge-separated

states; and (3) wavelength-dependent, subpicosecond experimental results, to test model predictions.

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