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The entanglement of excitation energy transfer and electron transfer in the reaction centre of photosystem II

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The primary processes of the photosystem II reaction centre involve both ultrafast energy and electron transfers. In this paper we present some of our recent studies aimed at experimentally distinguishing and characterizing these processes. These experimental studies demonstrate significant differences between the behaviour of this photosystem and that of the purple bacteria. We also consider the results of calculations of the primary processes of PS II, and find that these numerical studies have been successful not only in simulating existing experimental data, but in predicting the results of subsequent experimental studies which are also presented here.

Keywords: femtosecond spectroscopy; photosynthesis; photosystem II; purple bacteria; electron transfer; charge separation

1. Introduction

Almost all of the energy for biological function is ultimately derived from photosynthesis. There are a number of methods by which organisms convert solar radiation into stored chemical energy, but the most important of these involves the splitting of water into its constituent parts and the release of molecular oxygen. The photochemical splitting of water into oxygen, electrons and protons is achieved by the photosystem two (PSII) complex of green plants, and PSII is therefore responsible both for the oxygen in our atmosphere and for much of the Earth's biomass.

The process of splitting water is a complex one and involves at least 20 discrete electron transfer reactions. The overall process can be split into three components: light harvesting, charge separation and water splitting. In higher plants, light harvesting is performed largely by the antenna complex known as LHC2 (Kuhlbrant *et al.* 1994) plus a contribution from the 47 kDa and 43 kDa antenna proteins. The excitation energy is transferred from the light harvesting array to the reaction centre (RC) of PSII. At the reaction centre the excitation energy is converted into a chemical potential by a series of electron transfer reactions. The hole which is left behind is transferred into the oxygen evolving complex of PSII where the oxidizing potential created is used to oxidize a complex of water bound to a cluster of four manganese ions (Diner & Babcock 1996). This complete cycle is repeated four times in order to build up sufficient oxidizing power to split water and release molecular oxygen.

PSII is unique in a number of ways, and one of the most striking is its sensitivity to photoinduced damage (Barber & Andersson 1992). The $D1$ protein of the PSII reaction centre is one of the most rapidly turned over proteins in nature, and overall

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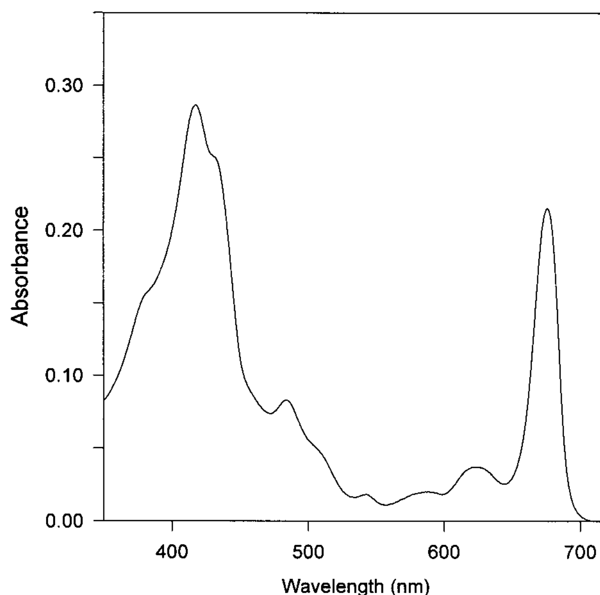

Figure 1. Absorption spectrum of the PSII reaction centre

PSII has numerous regulation and protection mechanisms which are not found in other photosystems. Some of these regulatory mechanisms are found at the level of the reaction centre. For example cyt-b559 acts as an electron switch being able to either receive electrons or donate them depending on the overall state of the system (Barber & De Las Ribas 1993). There are also changes which take place in the antenna of PSII to regulate the flow of excitation energy to the reaction centre (Horton *et al.* 1991). It seems likely that these protective mechanisms are a response to the damaging side reactions which accompany the production of an oxidizing potential which is sufficient to split water. Moreover the combination of oxygen and chlorophyll excited states is an unfortunate one, as it leads to the formation of highly reactive singlet oxygen which is quite capable of destroying PSII (Durrant *et al.* 1990).

In order to study this complicated sequence of events, it is helpful to break the complete PSII complex into its component parts and to study these individually. The minimal chemically functional unit of PSII is the isolated reaction centre. The isolated reaction centre contains six chlorophyll-a (chl) molecules, two molecules of pheophytin-a (ph) one to two β-carotene molecules and one haem. These are bound by the two major reaction centre polypeptides $D1$ and $D2$ and the small cytochrome binding proteins, which is why the complex is sometimes known as the $D1$–$D2$–cyt-b559 complex. The overall structure of the PSII reaction centre is not known, but $D1$ and $D2$ show high identity with the L and M subunits of purple bacterial reaction centres, the structures for which are known (Michel & Deisenhoffer 1988).

2. The rate of primary charge separation in PSII

It has been known for many years that the dominant time constant associated with primary charge separation in purple bacterial reaction centres such as *R. sphaeroides* and *R. viridis* is approximately 3 ps. In order to make a similar measurement in PSII

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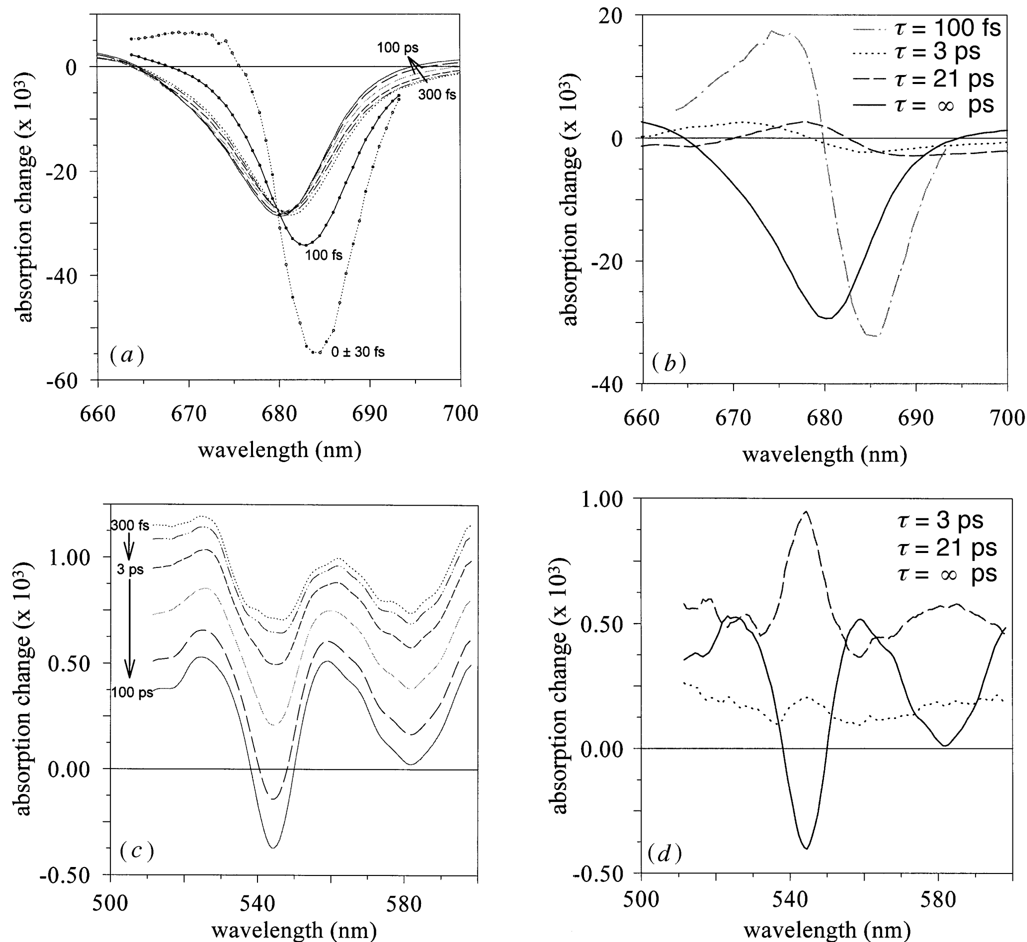

Figure 2. Transient absorption data from the Q_y ((*a*) and (*b*)) and Q_x ((*c*) and (*d*)) spectral regions of the PSII reaction centre. Panels (*a*) and (*c*) show some transient spectra taken at particular times. Panels (*b*) and (*c*) show the kinetic spectra associated with simultaneous non-linear least-square fits of 200 transient spectra. The time constants retrieved are shown in the top right-hand corner.

it is necessary to produce time-resolved spectra of the system in regions where the kinetics can be assigned.

One of the most awkward problems in studying PSII is that the absorption spectrum of the particle is highly congested, with the lowest optical transitions of the eight protein-bound chlorins lying within 10 nm of each other. This makes the assignment of kinetics very difficult using data from this spectral region alone. Unfortunately there are only two features which are unambiguously assignable in the PSII spectrum, and both of these are very small. Therefore in order to make assignable observations, it is necessary to collect data with a high signal-to-noise ratio in order to resolve the changes in the assignable regions of the spectrum.

The most easily assigned region is the ph Q_x band which is located at 545 nm. This weak band can be seen in the steady-state absorption spectrum of the isolated PSII RC shown in figure 1. The difference spectrum of the primary radical pair shows a characteristic shape in this spectral region which is dominated by the bleaching of

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the Q_x band. Time-resolved measurements show that the majority of this bleaching occurs with a 21 ps time constant, and that these spectral changes were accompanied by the production of a pheophytin anion band (Hastings *et al.* 1992). This observation demonstrates that most of the electrons arrived at the ph in 21 ps, but not the time constant with which the primary electron donor was oxidized. An example of this type of data can be seen in figure 2.

Measurements of oxidation rates have been made in purple bacterial RCs by time resolving the loss of stimulated emission from the particle. This is easily done in the case of purple bacteria, as emission from the singlet-excited state of the primary electron donor is strongly Stokes shifted to form a completely resolved emission band to the red of the lowest lying absorptive transition. The situation in PSII, however, is much less favourable. The Stokes shift in chl-a is about 6 nm in solution, and the emission from the PSII reaction centre peaks only 3 nm to the red of P680 at room temperature. This means that the primary electron donor emission is not resolved from the ground-state absorption. In order to time resolve the loss of singlet-excited states, it is necessary to find a region of a spectrum in which there is relatively little contribution from a ground-state bleach. In the case of PSII this means resolving the emission to a vibrational sideband which can just be seen in the emission spectrum at 725–735 nm. Our time-resolved measurements in this region of the spectrum suggested that 75% of the chlorin singlet stated were lost with a 21 ps time constant, with the other 25% apparently associated with a 3 ps time constant (Durrant *et al.* 1993).

We thus deduce that the dominant time constant associated with primary charge separation in the PSII reaction centre is 21 ps, seven times slower than in the case of the purple bacterial RC. Rapid charge separation is a requirement for efficient charge separation, and so at first sight this is a somewhat surprising result. The mechanisms and the reasons for this reduction in the effective rate of primary charge separation is therefore a matter of significant interest.

3. Excitation energy transfer

The isolation of the PSII reaction centre in 1987 (the $D1/D2$/cytochrome-b559 complex) has greatly facilitated study of its primary processes. The primary photochemistry of this complex involves both energy and electron transfer reactions which ultimately produce the radical pair state $P680^{+}Ph^{-}$. The kinetic scheme is sufficiently complex that the electron and excitation energy transfer processes are rather entangled.

(*a*) *Slow energy transfer*

We have previously shown that there are two pools of states in the isolated reaction centre which transfer excitation energy in about 100 fs (Durrant *et al.* 1992). These pools are named C670–676 and C680–684 as their peak absorbances occur at roughly these wavelengths. A number of other research groups have reported the presence of a slow energy transfer step of around 20 ps in the PSII reaction centre. It was therefore possible that the slow rate of primary charge separation was due to a slow energy transfer bottleneck. This can be shown not to be the case. When the 680 nm pool (C680–684) is photoselectively excited then the dominant time constant associated with primary charge separation is 21 ps. This lengthens to 27 ps when the 670 nm pool is excited. This difference in time constants in combination with the

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differences in the kinetic spectrum shows that the slow energy transfer observed by other groups does exist but does not cause the slow charge separation rate (Rech *et al.* 1994). If C680 selective excitation is dominated by excitation of P680 (the primary electron donor), then there can be no other energy transfer bottleneck in the system. Deconvolution of the PSII RC absorption spectrum suggest that C680 is dominated by P680 (Kwa *et al.* 1992; Otte *et al.* 1992; van der Vos *et al.* 1992), but there is at least one other state degenerate or nearly degenerate with P680. We have shown, however, that these two states transfer energy on a timescale of a few hundred femtoseconds and therefore that the degenerate state does not create a bottleneck. Moreover these energy transfer rates can actually be calculated (see below) and neither calculations nor data are consistent with extensive slow excitation energy transfer if the C680 pool is photoselectively excited.

We were also able to demonstrate that biochemical removal of one of the peripheral chlorophyll-a (chl-a) molecules reduced the extent of the slow energy transfer when C670 was excited. The removal of one of these peripheral chl-a, however, had no effect on the kinetics observed when C680 was excited (Vacha *et al.* 1995).

While there have been extensive studies of picosecond energy transfer processes associated with 'peripheral' chlorophylls bound to the exterior of the PSII reaction centre, studies of sub-picosecond energy transfer processes between the 'core' reaction-centre chlorins have to date been relatively limited. It is these sub-picosecond studies which demonstrate most clearly that there is little or no slow excitation energy transfer in the islolated PSII reaction centre when C680 is photoselectively excited. A description of these studies is given below.

(*b*) *Fast energy transfer*

A key feature which distinguishes the PSII reaction centre from the photosynthetic reaction centre of purple bacteria are the relatively small energy gaps which drive its photochemistry. These small energy gaps are most probably associated with the ability of PSII to generate a sufficiently high oxidizing potential to extract electrons from water, which imposes significant constraints upon the system. In particular, these small gaps result in extensive spectral overlap of the lowest electronic transitions (S_0 to S_1 or Q_y transitions) in the RC. In the purple bacterial reaction centre, the bacteriochlorin Q_y absorption maxima are distributed over 160 meV, with the primary donor being a deep energetic trap for excitation energy ($k_B T \sim 25$ meV at room temperature). In contrast, in the PSII reaction centre, the chlorin Q_y maxima are separated by no more than approximately 30 meV.

In our first study of sub-picosecond equilibration of excitation energy in isolated PSII reaction centres (Durrant *et al.* 1992), we concluded that equilibration of excitation energy between the majority of reaction centre S_1 excited states occurs with a time constant of 100 ± 50 fs, and prior to charge separation. Charge separation then proceeds from this equilibrated state (^{1}RC*) primarily with a time constant of 21 ps (Klug *et al.* 1995).

More recently, however, we demonstrated that a previously observed time constant of 400–600 fs is also the result of fast energy transfer. In this case, however, the excitation energy transfer is between two degenerate states at 680 nm (Merry *et al.* 1996).

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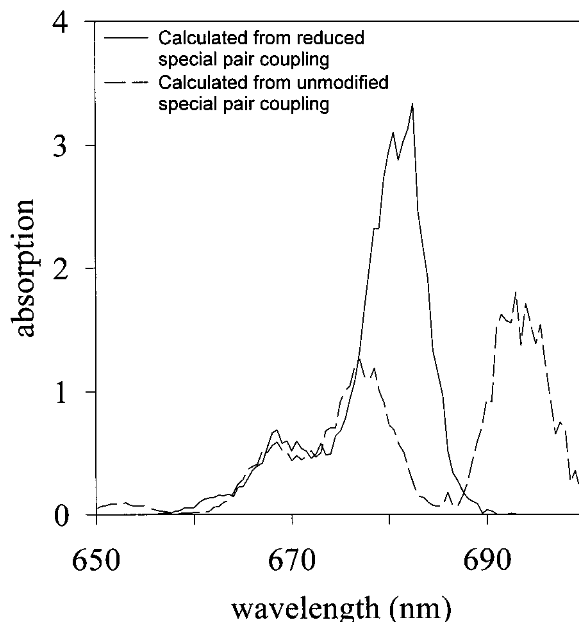

Figure 3. Calculated absorption spectra of the PSII reaction centre.

4. The nature of the primary electron donor: an excitonic chlorin multimer

In reaction centres of purple bacterial, the primary electron donor is formed by a pair of chlorophyll molecules which are strongly dipole–dipole coupled. This means that charge separation proceeds from a delocalized exciton state. Despite similarities in structure, the situation in PSII appears to be quite different.

There have been a number of suggestions that P680 (the primary electron donor in PSII) is a special pair, by analogy with the structure of the RC of purple bacteria. However, a number of observations have led to the conclusion that the special pair in PSII is more weakly coupled than in the purple bacterial RC and that structural models which suggest that P680 is a dimer are misleading. In fact the coupling strength from any putative special pair appears to be of the same order as that between the other cofactors. This can be inferred either from the rate of energy transfer between singlet states or other observations of the coupling such as circular dichroic absorption.

The steady-state optical properties of the PSII reaction centre can be calculated by considering the effects of dipole–dipole coupling between the optical transitions of the chlorins embedded in the protein matrix. Inhomogeneous broadening is taken into account by using Monte Carlo simulations and allowing each chlorin to have gaussian disorder in its transition energy. These simulations show that the similarity in coupling strengths and transistion energies has the somewhat surprising effect of causing the singlet-excited states to delocalize to a far greater extent than would be the case if one pair of cofactors were significantly more strongly coupled than the rest (Durrant *et al.* 1995*b*). Extensive delocalization seems to occur even in the presence of significant inhomogeneous broadening. The value for this inhomogeneity is taken as 240 cm^{-1} (FWHM), which is consistent with the results of hole burning

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measurements, and yet this value still allows the average singlet-state delocalization to be over 2–3 pigments.

It is clear from this type of analysis that the singlet-state coupling in PSII produces significantly different behaviour from that found in purple bacterial RCs. One of the two spectra shown in figure 3 is calculated by assuming that the tightly coupled pigments in the PSII RC are arranged in the same way as those in the PB RC. The other spectrum is calculated by reducing the strength of coupling of the 'special pair' chl molecules to be consistent with circular dichroism and energy transfer measurements. This reduction in coupling can be achieved in many ways but the effect on the spectrum is essentially the same. In the case of unmodified coupling, the spectrum obtained is very different from that found in the real system, while in the reduced coupling case the spectrum is similar to that which one would obtain if the weakly coupled 'peripheral' chls were absent.

The most surprising thing about the above discussion is that calculations suggest that singlet-excited states (exciton states) of the reaction centre may be delocalized over both the primary electron acceptor (a ph molecule) and primary electron donor (P680), and that primary charge separation may therefore be actually *intra*-molecular rather than *inter*-molecular as in purple bacterial RCs. In any case the conclusion from this analysis is that in general the singlet-excited states of P680 are likely to be delocalized over more than two pigments in the RC and that P680 is therefore a multimer of relatively weakly coupled pigments rather than a special pair as in the PB RC. Although it seems that the singlet states may be extensively delocalized, this does not imply that the same is true of the cation and anion states. Even though the cation and anions states are localized, their creation still causes a bleaching of the P680 transition. This means that the Q_y difference spectra of all of the excited/ion states of the system are likely to appear as very similar, which is consistent with experimental observation (see figure 1) (Klug *et al.* 1995).

In the multimer model of PSII, the combination of delocalization and inhomogeneous broadening makes it difficult to relate spectral features to sub-structures of the chlorophyll protein complex. It is, however, possible to unpick the Monte Carlo simulation to gain some insight into the energy transfer processes which occur. We find that the two degenerate redmost states can happily coexist with the bluer states on the same arms of the reaction centre and therefore that the 100 fs energy transfer process corresponds to energy transfer within one arm of the reaction centre. We have found, however, that the two degenerate redmost states (680–684 nm) correspond on average to optical transitions which are localized either on one arm of the reaction centre, or on the other arm, and do not in general show much common contribution from a given pigment (Merry *et al.* 1996). This suggests that the 400–600 fs energy transfer rate between these two states corresponds to energy transfer between the two arms of the reaction centre.

Calculations of the transient absorption spectra which one would expect are also quantitatively consistent with observations. Table 1 compares the results of calculations to the experimentally determined parameters. The agreement is really rather good and provides support for the multimer model of the singlet states in the PSII reaction centre.

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Table 1. *Optical properties of the two long-wavelength transitions (680–684 nm) in the isolated PSII reaction centre*

	multimer model calculations	results from femtosecond transient absorption spectroscopy
average oscillator strength (relative to monomeric chl-a)	1.9	1.9 ± 0.5
average ratio of oscillator strengths	1.7[a]	1.8 ± 0.2
average angle between optical transitions	80	70 ± 10

[a]The average ration of oscillator strength is taken as (oscillator strength of the highest oscillator-strength transition over the oscillator strength of the second highest oscillator-strength transition).

5. Calculating the rates of excitation energy transfer

It is rather straightforward to write down some equations of motion for transport of the singlet-excited states in a system such as the PSII multimer. Unfortunately these equations of motion cannot be solved except in certain limits.

We have recently derived expressions for the energy transfer dyanamics of the PSII exciton states (Leegwater *et al.* 1997). The transport mechanism which we describe is a generalization of the Förster transfer mechanism as it holds irrespective of the strength of the dipole–dipole coupling, relative to the intensity of the line-broadening mechanisms (Leegwater *et al.* 1997) and is therefore valid for calculating the rates of excitation energy transfer between exciton states. The theory which we apply is based on the assumption that the exciton–phonon interaction is fairly weak and is local to the sites which are occupied by the cofactors of the reaction centre. In our picture, the phonons cause a time-dependent shift of the individual chlorin's transition energy and hence they allow excitation energy to be transferred between states of different frequency.

We are interested in the rate of change of the exciton populations, which are the diagonal elements of the time-dependent reduced density matrix:

$$\frac{\mathrm{d}}{\mathrm{d}t}\rho_{k,k}(t) = -\sum_{k_1} \Gamma_{k,k;k_1,k_1}\rho_{k_1,k_1}(t), \tag{5.1}$$

where k and k_1 are exciton states, and the above equation refers to the rate of change of the population of the exciton state k.

By assuming that the electron–phonon coupling is Markovian, we end up with the following expression for the rate of transfer between exciton states:

$$\Gamma_{k,k;k_1,k_1} = 2\delta_{k,k_1}\sum_{i,K}\gamma(\omega_{k_1}-\omega_K)|\langle i|\psi_K\rangle|^2|\langle i|\psi_{k_1}\rangle|^2 - 2\sum_{i}\gamma(\omega_{k_1}-\omega_k)|\langle i|\psi_k\rangle|^2|\langle i|\psi_{k_1}\rangle|^2. \tag{5.2}$$

In the above expression, i is the index of the molecular states rather than the exciton states while ψ_K and ψ_{k_1} are wavefunctions of the exciton states. $\gamma(\omega_{k_1}-\omega_K)$ includes detailed balance and is the term describing the strength of the electron–phonon coupling and the phonon spectrum.

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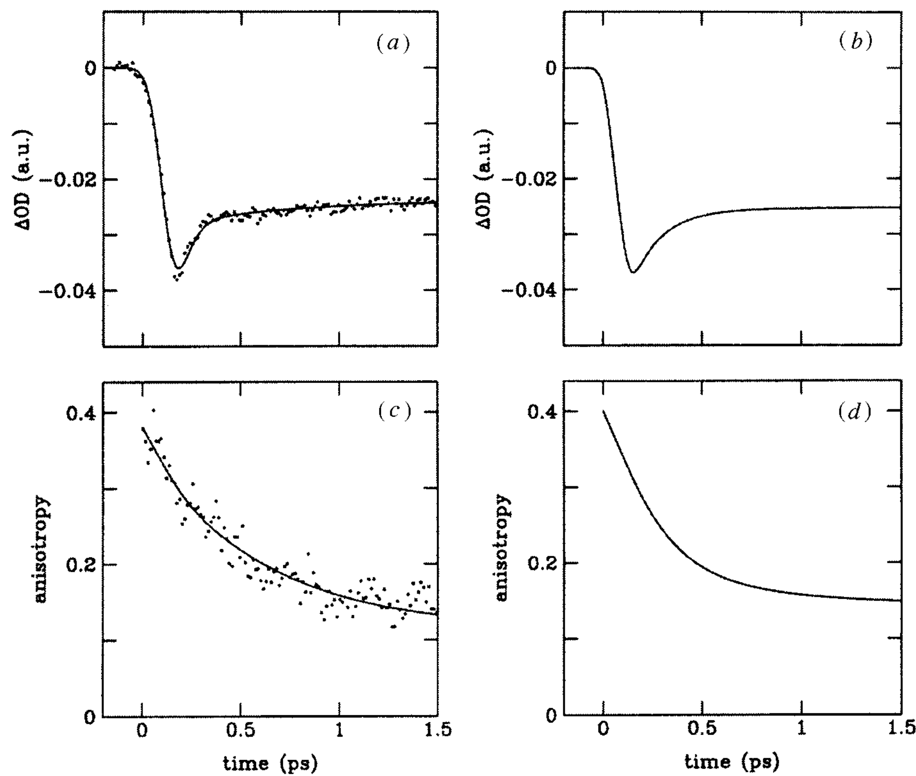

Figure 4. Rates of excitation energy transfer within the PSII multimer. Comparison of the results of experimental studies ((*a*) and (*c*)) with the results of numerical simulations. The transient absorption data were collected at a detection wavelength of 683 nm following excitation of the redmost exciton states. (*a*) and (*b*) show the isotropic signal; (*c*) and (*d*) show the transient anisotropy.

It can be seen from equation (5.2) that the rate of transfer between exciton states is strongly dependent on the extent to which exciton states share molecular sites. The greater the commonality between states (k and k_1) via the monomer sites (i), then the greater the probability of a site local phonon causing a transition, by either delivering or carrying away the energy difference between the exciton states.

A comparison of the rates of excitation energy transfer calculated using equation (5.2) above and those determined experimentally is shown in figure 4.

The calculations and experimental data agree reasonably well considering the assumptions which are required to derive equation (5.2). As with the contents of table 1, the implication of figure 3 is that the isolated PSII reaction centre does behave like a disordered supermolecular system.

6. Electron transfer

In the isolated PSII reaction centre (the $D1/D2$/cytochrome-$b559$ complex) formation of this primary radical pair $\mathrm{P680^+Ph^-}$ is preceded by extensive and rapid (less than 1 ps) equilibration of excitation energy between the reaction centre chlorins (see above). This equilibration is expected to cause the experimentally observed time constant for charge separation in this complex to be significantly slower than the underlying intrinsic rate constant for this process. For example, if the excita-

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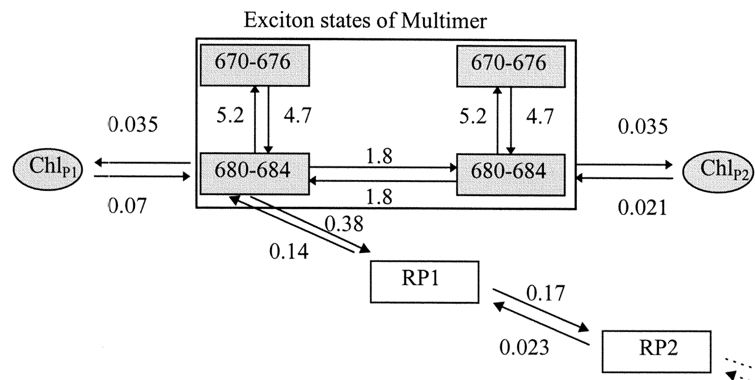

Figure 5. A kinetic model of energy and electron transfer pathways within the isolated PSII reaction centre, with the rate constants given in ps^{-1} (error margins ±20%). The model includes the sub-picosecond energy transfer within the P680 multimer and slower energy transfer processes associated with peripheral chlorophylls (chl_{P1} and chl_{P1}). As previously, each of the multimer state absorbing 670–676 nm are assumed to be two-fold degenerate. In addition, the model includes two sequential radical pair states RP1 and RP2. These states correspond to unrelaxed and relaxed forms of the radical pair $P680^{+}Ph^{-}$.

tion energy were equally equilibrated between all eight reaction centre chlorins, this would result in an eight-fold increase in the observed time constant for the charge separation process in PSII (Durrant *et al.* 1992; van Grondelle *et al.* 1994).

Primary charge separation in reaction centres of the purple bacterium *Rhodobacter* (*Rb.*) *sphaeroides* is not mono-exponential, although the dominant time constant in this case is about 3 ps rather than approximately 20 ps as observed for PSII (Holzapfel *et al.* 1990). There is much evidence that primary radical-pair formation if the reaction centres of *Rb. sphaeroides* proceeds via an intermediate state in which the electron resides on an accessory chlorophyll (Holzapfel *et al.* 1990). The exact nature of this intermediate state is still a matter of debate, but the action of this state is largely responsible for a component of about 1 ps. Other components have also been reported with lifetimes of tens to hundreds of ps, although these only account for a small proportion of the charge separation (Woodbury *et al.* 1994; Du *et al.* 1993). These components have been ascribed either to inhomogeneous distributions of radical-pair states (Ogrodnik *et al.* 1994) or to relaxations of the protein matrix (Du *et al.* 1993). Indeed, it has been suggested that the charge separation process in the *Rb. sphaeroides* is adiabatic, and rate limited by vibrational relaxation of the protein matrix (Lin *et al.* 1996).

Our own studies (Hastings *et al.* 1992; Durrant *et al.* 1993; Klug *et al.* 1995), and more recently those of Sension and co-workers (Donovan *et al.* 1996) have concluded that $P680^{+}Ph^{-}$ formation primarily proceeds with a time constant of 20 ps. However, the detailed consideration of the kinetics of formation of this state, and the concomitant decay of reaction-centre singlet-excited states, show large deviations from mono-exponential behaviour (Durrant *et al.* 1993; Muller *et al.* 1996; Greenfield & Wasielewski 1996). Our own studies have, for example, indicated that additional 3 and 100 ps components account for approximately 26% and 15% of emission decay, respectively (Klug *et al.* 1995; Kumazaki *et al.* 1995).

The simplest reaction scheme which can account for our experimental observations is shown in figure 5.

Slow energy transfer from the peripheral chlorophyll molecules can be avoided by direct excitation of the redmost states (680–684 nm) of the PSII multimer. Under

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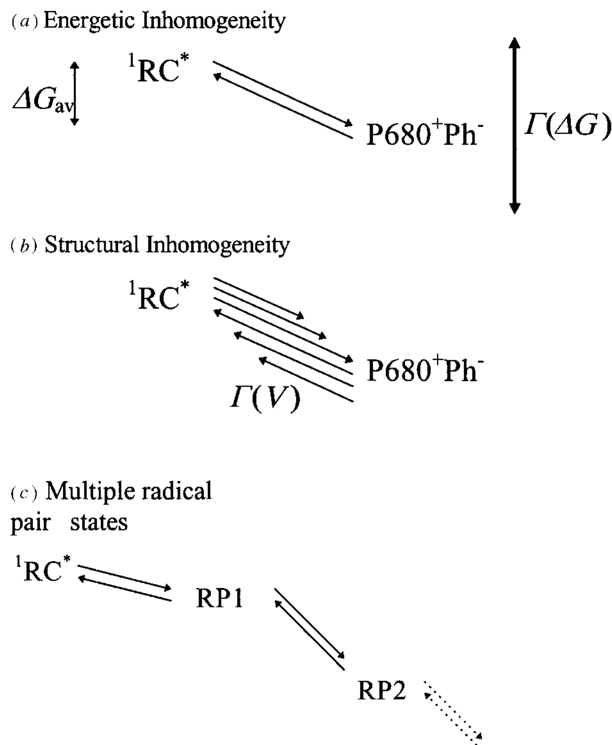

Figure 6. Possible origins of the multiphasic kinetics observed for radical pair formation in PSII: (*a*) static inhomogeneity in energy; (*b*) static structural inhomogeneity (inhomogeneity in donor–acceptor separation); (*c*) intermediate steps in the trapping process.

these conditions the observed kinetics can be essentially summarized as:

$$^1\mathrm{RC}^* \underset{0.15}{\overset{0.09}{\rightleftharpoons}} \mathrm{RP1} \underset{0.026}{\overset{0.14}{\rightleftharpoons}} \mathrm{RP2}.$$

Under conditions where the redmost states of the PSII multimer are excited, there are essentially three possible explanations for the multiple time constants associated with primary charge separation in the PSII reaction centre (see figure 6): (i) the kinetics may be the result of inhomogeneity in the energies of either the primary donor state and/or the radical pair state; (ii) there may be inhomogeneity in the electronic coupling constant between the donor and acceptor molecules; and (iii) there may be intermediate steps on the path to the final radical-pair state.

(*a*) *Energetic inhomogeneity*

If we assume that the electron transfer reactions are non-adiabatic, then we can employ conventional electron transfer theory to model the rates of reaction as a function of the energy gap ΔG between ^{1}P680* and P680$^+$Ph$^-$. A simple Monte Carlo simulation was employed to simulate the influence of a static Gaussian distribution for ΔG (FWHM Γ, mean ΔG_{av}) upon the experimentally observed kinetics for radical-pair formation at room temperature. These simulations included the influence of ΔG on the forward and reverse electron transfer rate constants and the final equilibrium constant. (Further details of this simulation can be found in Durrant *et al.* (1995*a*).) This simulation predicts transient kinetics which exhibit only small deviations from mono-exponential behaviour.

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Qualitatively similar behaviour was obtained for a range of different values of $\Delta G_{\rm av}$, λ (the reorganizational energy) and Γ: simulations employing: $0 < \Delta G_{\rm av} < 0.15$ eV, $0.05 < \lambda < 0.25$ eV and $0 < \Gamma < 0.1$ eV were all unable to yield a reasonable fit to the experimental data. Similar results were also obtained for simulations in which energy-transfer processes associated with the peripheral chlorophylls were explicitly included (see also below). The range of values employed for $\Delta G_{\rm av}$, λ and Γ include all reasonable values for these parameters—previous experimental studies have indicated $\Delta G_{\rm av} \sim \lambda \sim 0.06$–$0.11$ eV and $\Gamma \sim 0.06$–0.1 eV. Deviation from mono-exponential behaviour comparable to that observed experimentally was only found for values of $\lambda < 0.5\Delta G_{\rm av}$ and $\Gamma \sim 0.1$ eV. However, these small values of the reorganizational energy would be inconsistent with the observation of a high quantum yield of charge separation in PSII at 77 K (Groot *et al.* 1997). Assuming that non-adiabatic electron transfer theory is appropriate to apply to these systems, we are forced to conclude that a static inhomogeneity in the energy gap associated with primary charge separation is insufficient to generate the extreme multiexponential charge-separation kinetics observed for PSII at room temperature.

(*b*) *Structural inhomogeneity*

Another possible explanation is inhomogeneity in electron transfer coupling constants due to structural inhomogeneity. Monte Carlo simulations similar to those employed above were conducted to evaluate the effect of a Gaussian distribution (FWHM Δr) of donor/acceptor separation r upon the charge separation kinetics, assuming the electron transfer rate is proportional to $e^{-\beta r}$ and $\beta = 1.6$ Å^{-1} (Moser *et al.* 1992). These simulations yielded kinetics which deviated only marginally from mono-exponential behaviour for $r_{\rm av} \sim 5$–10 Å and $\Delta r \leqslant 2$ Å. Indeed values of $\Delta r \sim 4$ Å are required to produce multiexponential kinetics comparable to those observed experimentally. Variations in pigment separation as large as 4 Å, corresponding to at least a 40% change in distance, seem implausible given the crystallographic data determined for the bacterial reaction centres. It thus appears that structural inhomogeneities are unlikely to account for the multiexponential charge separation kinetics observed for PSII reaction centres.

(*c*) *Multiple radical pair states*

Having discounted alternative causes of the multiexponential charge separation kinetics observed for PSII, we have to address the possibility that these kinetics result from multiple steps in the charge separation process in PSII. Figure 5 illustrates such a kinetic model, in which we have extended our previously published kinetic model for energy-transfer processes within the PSII reaction centre by the inclusion of a sequence of two charge separated states RP1 and RP2. This model is sufficient to account for the experimentally observed 3 and 21 ps charge-separation components.

Consideration of the forward/reverse rates determined for the kinetic model illustrated in figure 5 allows determination of the relative energies of states involved. The initially formed RP1 state (i.e. $\mathrm{P680^+Ph^-_{unrelaxed}}$) is approximately 25 meV below each of the redmost states of the P680 multimer (i.e. approximately isoenergetic with $^1\mathrm{RC^*}$), while the RP2 state ($\mathrm{P680^+Ph^-_{relaxed}}$) lies approximately 50 meV lower in energy. The 3 ps phase of charge separation is primarily associated with equilibration of the chlorin singlet states with an unrelaxed $\mathrm{P680^+Ph^-}$ state. Subsequent relaxation of $\mathrm{P680^+Ph^-}$ accounts for the dominant phase of formation of the radical pair and occurs with a 21 ps time constant. This model could be readily extended to

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further energetic relaxation of the $P680^+Ph^-$ state to take account of the putative approximately 100 ps phase of charge separation, although further data are needed before this component can be unambiguously assigned.

It should be pointed out that these results do not rule out the role of a $P680^+chl^-$ state in the charge separation process in PSII but merely indicate that the multi-exponential charge separation kinetics observed to date do not originate from the presence of such an intermediate state.

Relaxation models such as the one described here have been suggested as an explanation for the multiple time constants for primary charge separation which are found in the reaction centre of purple bacteria. However, because of the smaller free energy loss associated with charge separation in PSII, the effect of these relaxations are enhanced in this photosystem and therefore play a critical role in stabilizing the charge-separation process. These relaxation processes occur over a wide range of time scales. At approximately 60 ps the energy gap between the $^1RC^*$ and $P680^+Ph^-$ is only approximately 50 meV, with further relaxations accounting for the 100 ps (and slower (Booth *et al.* 1991)) components, ultimately resulting in an energy gap of approximately 110 meV on the ns timescale.

(*d*) *Intrinsic rate of charge separation*

The kinetic model shown in figure 5 indicates that the intrinsic rate constant for charge separation from one excited state of the P680 multimer to the initially unrelaxed $P680^+Ph^-$ is $(0.38 \pm 0.1)\ ps^{-1}$. However, considerable caution should be used in consideration of this rate constant. First, this process is associated with essentially no loss of free energy and therefore does not trap much excitation energy from reaction-centre singlet-excited states. Trapping of singlet-excited states by radical-pair formation exhibits multiexponential behaviour, with the dominant phase, exhibiting a $(0.17 \pm 0.3)\ ps^{-1}$ rate constant, which we associate with protein relaxation processes. Second, the extent of charge separation to the initial unrelaxed $P680^+Ph^-$ state depends critically upon the energy of this state. In larger PSII complexes, with more subunits present, the energy of this state could be modified by the protein/membrane environment of the reaction centre, preventing a simple extrapolation of the results in isolated reaction centres. Finally it should be noted that there is no evidence that charge separation in PSII is associated with electron transfer from a single excited state to a single radical-pair state. As we have previously discussed, the reaction-centre singlet states can most probably be described as delocalized exciton states resulting from a multimer of reaction centre chlorins (Durrant *et al.* 1995*b*). As such the charge separation process could be described as the stabilization of charge-transfer states within a supramolecular complex. Indeed, it has recently been proposed that in certain mutants the charge separation in purple bacterial reaction centres may proceed directly from singlet-excited states of the accessory chlorophylls without the involvement of the low energy special pair exciton state (van Brederode *et al.* 1997). Given the greater degeneracy of the excited states in PSII, parallel pathways for charge separation are likely to be even more prevalent in this photosystem.

(*e*) *Adiabatic electron transfer*

The data we have presented here indicates that formation of the radical-pair state on the ps timescale is multiphasic and appears to be rate limited by relaxation processes of the protein. It should also be noted that Stark spectroscopy of the PSII reaction centre indicates that optical excitation of P680 multimer excites states with

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a significant degree of charge-transfer character (Steffan 1994). The role of charge-transfer states of the special pair of the bacterial photosystem has been widely discussed (see, for example, Lin *et al.* 1996; Lathrop & Friesner 1994), and recently analogous charge transfer states of the P680 multimer have been suggested to be important for the charge-separation process in PSII (Groot *et al.* 1997). It is possible therefore that for PSII the charge-separation process is initiated directly upon excitation of the P680 multimer, within the temporal evolution of the system being dominated by protein relaxation processes which result in the stabilization of the charge-separated states.

We conclude that the charge separation kinetics in isolated PSII reaction centres exhibit a much larger deviation from mono-exponential behaviour than those observed for reaction centres isolated from purple bacteria. This difference is associated with the smaller free-energy gap between singlet-excited states and radical-pair states in this reaction centre. On the basis of numerical simulations we conclude that this deviation of mono-exponential behaviour can not result solely from static energetic or structural inhomogeneities. It should be noted that our results are not inconsistent with such inhomogeneities, we only conclude that these are insufficient to account for the multiexponential charge-separation behaviour observed for this reaction centre. Our results are most consistent with a relaxation of the radical-pair free energy. This relaxation appears to have a strong effect on the primary trapping of excitation energy within the PSII reaction centre.

7. Conclusion

The key features of the electron and energy transfer reactions in the isolate PSII reaction centre are summarized as follows.

(1) The six core chlorins of the reaction centre interact via dipole–dipole coupling to create a set of disordered exciton states. The spectrum of the reaction centre can be calculated by taking this into account.

(2) Energy transfer between the exciton states occurs broadly on two timescales: 100 fs for transfer between red and blue states on one arm of the RC; and 400–600 fs for transfer between the two arms which corresponds to transfer between the redmost states of the system.

(3) The relative rates of energy transfer between the exciton states is reasonably well represented by calculations which assume Markovian electron–phonon coupling and take the site energy disorder of the chlorins into account.

(4) Slow energy transfer from the two peripheral chlorophylls does not occur if red photoselective excitation is used thereby exciting only the redmost exciton states of the RC at 680 nm. Slow energy transfer does contribute to the overall dynamics, however, and strongly so if the blue states at 670 nm are excited.

(5) Formation of the primary radical pair is dominated by a 20 ps time constant but there is a minor contribution from a 3 ps component. This slow effective electron transfer rate is largely due to the low population of the P680 singlet state due to extensive uphill energy transfer which occurs even if C680 is selectively excited prior to electron transfer.

(6) The non-exponential formation of the primary radical pair can only be explained by the formation of an intial radical-pair state which is almost isoenergetic with the P680 excited-singlet state and the subsequent relaxation of the radical pair on a 20 ps tiemscale. This means that most of the trapping of excitation energy

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in the isolated PSII reaction centre occurs via relaxation of the chromophore–protein complex.

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