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Energy transfer, charge separation and pigment arrangement in the reaction center of Photosystem II

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Abstract

Energy transfer and charge separation in the isolated Photosystem II reaction center at room temperature were studied with transient absorption difference spectroscopy upon selective excitation of the reaction center pigments. The measurements were performed with two dye lasers, which had a spectral bandwidth of less than 1 nm, and with an instrument response function of 5 or 18 ps depending on the type of experiment. Small changes with time constants of 0.6 ns and 120 ps are attributed to damaged reaction centers. Selective excitation of the long-wavelength pigments, presumably P680 and the pheophytins, led to charge separation in 3 ps. Selective excitation of the short-wavelength pigments, presumably accessory chlorophylls, led to charge separation in 30 ps with the same quantum efficiency. This excludes equilibration of the excited state between accessory chlorophyll and P680 in less than 30 ps. The overlap of the fluorescence spectrum of accessory chlorophyll with the absorption of P680 is very good and the slow energy transfer is attributed to an about 30 Å center-to-center distance, which makes the histidines 118 in helix II of the D1 and D2 proteins likely binding sites of the chlorophylls nearest to the long-wavelength pigments, P680 and pheophytin. Reevaluation of the literature in the light of these data suggests that P680 is a dimer with nearly (anti) parallel Q_Y-transition moments of the constituent monomers, making an angle with their connecting axis close to the magic angle, and that the geometry of P680 and the pheophytins is not C_2-symmetrical around an axis perpendicular to the membrane.

Key words: Picosecond transient absorption; Energy transfer; Charge separation; Reaction center; Photosystem II; Pigment arrangement

1. Introduction

Energy transfer and trapping in the isolated reaction center of Photosystem II (PS II RC) has been studied by several groups in the past few years, but a consensus picture has not yet emerged. Part of the problem may have been the instability of the early preparations and variability of their pigment composition. Current isolation procedures [1,2] result in particles containing 6 chlorophylls *a* (Chls) and 2 pheophytins *a* (Pheos) per RC [2–4], but there are indications that the original isolation procedure of Nanba and Satoh yields fewer Chls per Pheo [5–8] and it has been reported that extensive washing with Triton X-100 selectively removes 2 of the 6 Chls [9]. The main difficulty, however, is that all these pigments have very similar absorption spectra. At room temperature the red absorption band, centered at 675 nm, does not show any structure. The primary electron donor P680 accounts for a substantial part of the absorbance around 680 nm and the accessory Chl accounts for most of the absorbance around 670 nm [10–12]. The Pheo which acts as the primary electron acceptor most likely has its absorption maximum near 677 nm [12]; indications for a Pheo absorption band at longer wavelength have also been reported [13,14].

In the isolated PS II RC, the primary radical pair $P680^+Pheo^-$ cannot reduce the secondary electron acceptor, plastoquinone Q_A, because it is lost during the isolation, and it also does not oxidize the secondary electron donor, tyrosine Z, perhaps because $Pheo^-$

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Abbreviations: Chl, chlorophyll *a*; P680, primary electron donor; Pheo, pheophytin *a*; PS II, Photosystem II; RC, reaction center.

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must be reoxidized first [15]. Consequently, $P680^+$ $Pheo^-$ decays by charge recombination. At room temperature this takes about 35 ns [16] and the triplet yield is about 25–30% [17,18]. Under anaerobic conditions the lifetime of the triplet state of the primary donor, $P680^T$, is 1 ms [18].

In spite of the small number of pigments and very limited photochemistry at least 5 different kinetic phases have been detected by time-resolved spectroscopy in the ps and sub-ps time domain. The time constant of charge separation is still subject to discussion. In intact PS II, efficient photosynthesis requires that it is much less than 20 ps, because the excited state lives about 2 ns when no charge separation takes place and should spend only about 1% of this time on P680 if the distribution of the excited state is thermally equilibrated [19]. A time constant of 3 ps was predicted on the basis of time-resolved fluorescence measurements on a PS II core preparation [20]. Early fluorescence studies on the isolated PS II RC led to proposals that charge separation takes 25 ps [21] and that none of the fluorescence decay phases reflect charge separation [22].

The first study with adequate time resolution (500 fs) was performed by Wasielewski et al., who concluded from transient absorption measurements that after rapid energy transfer to P680 the charge-separated state was formed in 3.0 ps [23] and 1.4 ps [24] at 277 K and 15 K, respectively, and a 25 ps (or 12 and 50 ps) component was attributed to an energy transfer process which is not connected with the primary charge separation [24,25]. These results were in good agreement with hole-burning data obtained at 7 K by Tang et al. [13], who reported a 1.9 ps lifetime of P680*, and energy transfer from Chl to Pheo in 12 ps and from Pheo to P680 in 50 ps.

Time-resolved fluorescence experiments by Holzwarth and coworkers at 277 K and 77 K on RCs containing 4–5 Chls [26], and at 277 K on RCs containing 6 Chls [27] resulted in the resolution of several kinetic components, of which the most rapid three had lifetimes of 3, 34 and 140 ps. The 3 ps component was attributed to charge separation, while the 34 ps component was assigned to energy transfer from accessory Chl to P680. The 140 ps component was thought to represent a charge recombination process. In a low temperature study on RCs containing 6 Chls the same authors [28] found energy transfer times comparable to those reported by Tang et al. [13].

In transient absorption measurements by Durrant et al. [29] and Hastings et al. [30] four kinetic components were observed with lifetimes of 0.4, 3.5, 21 and 200 ps. The two most rapid components were attributed to decay of an initially delocalized P680 singlet excited state, the 21 ps component was assigned to the charge separation process, and the 200 ps component to a slow energy transfer/trapping process. Recently, Durrant et al. [31] reported the observation of an even faster kinetic component with a lifetime of 0.1 ps. The sign of this component depended on the excitation wavelength and therefore it was attributed to energy equilibration between two pigment pools.

Freiberg et al. [32] reported fluorescence lifetimes of 13 and 110 ps in PS II RC at room temperature. The 13 ps component was attributed to trapping of the equilibrated state into the primary radical pair state, while the 110 ps component was attributed to recombination luminescence.

The lack of consensus on the time constants involved in energy transfer and trapping in the PS II RC might be due in part to the different excitation wavelengths and bandwidths used by the different research groups. Here we present the results of time-resolved absorbance measurements at room temperature in the Q_Y-absorption region using two synchronously pumped dye lasers with a spectral bandwidth of less than 1 nm, which allowed selective excitation of the different pigments. Initially we did not detect a 3 ps component [33], but improvement of the sensitivity and extension of the spectral range have changed that. The main conclusions are that charge separation upon P680 excitation takes 3 ps and energy transfer from accessory Chl to P680 takes 30 ps. The absence of rapid equilibration of the excited state between accessory Chl and P680 has important implications for the interpretation of other kinetic and spectral data, and sheds new light on the structure of the PS II RC.

2. Materials and methods

PS II reaction centers were isolated from spinach according to [2] and stored in the dark at 77 K. Before the measurements the samples were thawed and diluted to an absorbance of about 0.7 mm^{-1} at 675 nm. In order to obtain anaerobic conditions in the sample, catalase, glucose, and glucose oxidase were added, in that order, to final concentrations of 120 μg/ml, 10 mM, and 120 μg/ml, respectively. All measurements were done at 295 K.

The transient absorption experiments were performed on an apparatus consisting of two synchronously pumped dye-lasers [34]. This set-up could be used in either a one- or two-color pump-probe mode with an instrument response function of $\sim$ 5 ps and $\sim$ 18 ps, respectively. In the former mode only one dye-laser was used, so that samples were excited and probed at the same wavelength, while in the latter mode both dye-lasers were used and probing and excitation wavelengths could be varied independently. The pump and probe pulses were polarized parallel. The laser for the one-color experiments was operated with

the dye DCM (4-(dicyanomethylene)-2-methyl-6-(*p*-dimethylaminostyryl)-4*H*-pyran) and was also used as excitation laser in the two-color mode, while the probing laser in this mode was run with the dye LDS 698 (Pyridine 1). Fits to the measured kinetics were convolutions of exponential decays with the instrument response function, which was described by a secanthyperbolic or a gaussian function with an appropriate width, in case of the one-color or two-color experiments, respectively.

The duration of a single laser-pulse was about 2–3 ps with a spectral bandwidth of less than 1 nm allowing selective excitation of the various RC pigments. Since the repetition rate of the dye-lasers is 76.6 MHz, the sample was placed in a rotating sample cell with a radius of 55 mm in order to avoid accumulation of closed RCs. Rotating the cell with 3000 rpm, and taking into account the spot diameter of the excitation beam of 40 μm, a sample volume collected 80 pulses during one pass through the excitation beam. The time between two successive passages of the same volume through the excitation beam, 20 ms, was long enough for the RCs to relax to their ground states. At the start of each measurement the sample cell was shifted in a direction perpendicular to the excitation beam, so that a fresh part of the sample would be illuminated. The average excitation power was typically 5 mW, which is about 65 pJ/pulse, and the power of the probe beam was at least 20-times less than that of the excitation beam. An excitation energy of 5 mW and a total absorbance of 1 at 675 nm resulted in excitation of 1% of the RCs per pulse.

With a triplet yield of 0.3 accumulation of the triplet state, $P680^T$, by the 80 pulses occurred in about 20% of the reaction centers during the passage through the beam, implying that with 675 nm excitation, on the average about 10% of P680 was in the triplet state before the flash. Excitation at wavelengths where the absorbance is lower should accumulate correspondingly less $P680^T$. It was verified that the kinetic shape of the signals was not significantly different at 10-fold lower excitation energy.

On changing the wavelength of measurement the sample cell was shifted in the direction of the measuring beam to restore maximum overlap of excitation and measuring beam in the sample, using the signal amplitude as a criterion. For this reason, there is some scatter in signal amplitudes and precise spectra of the measured absorbance changes cannot be given.

3. Results

Fig. 1 shows absorbance changes induced by excitation of PS II RC particles at a series of wavelengths in the Q_Y-absorption region as measured at the wavelength of excitation. At all wavelengths an initial bleaching is observed, followed by an absorbance increase which is strongly wavelength-dependent, both in relative amplitude and in kinetics. Around the 675 nm absorption maximum of the particles the initial bleaching is largely irreversible on the 200 ps timescale measured. At shorter wavelengths, where the absorbance is largely due to accessory Chls, it is followed by an increase with a time constant of 25–30 ps; at longer wavelengths, where P680 and perhaps Pheo absorbance is expected, by an increase with a time constant of 3 ps (the traces at 679 and 682 nm actually show a mixture of both phases). The apparent amplitude of the 3 ps component is suppressed about 3-fold by convolution with the instrument response function. Presumably, the initial bleaching reflects the disappearance of ground state absorption and – especially at longer wavelengths – appearance of stimulated emission from the excited state formed, and the absorbance change remaining after 200 ps is due to the formation of the radical pair. In between, energy transfer to other pigments and charge separation may be responsible for the observed absorbance increase. Since the increase of the absorbance is 10-times slower upon excitation of the accessory Chls than upon selective excitation of P680, the most simple interpretation would be that energy transfer from accessory Chls to P680 takes 30 ps and excited P680 leads to charge separation in 3 ps.

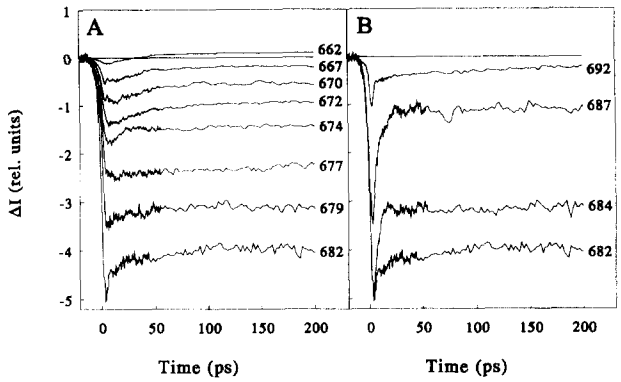

Fig. 1. Absorbance changes of the PS II RC measured in the one-color pump-probe mode in the wavelength region where mainly accessory Chls absorb (A) and where mainly P680 absorbs (B). All the measurements have been normalized on the number of absorbed quanta and the intensity of the probe beam. The point of zero-delay was obtained from the fitting procedure. The average excitation energy was about 5 mW.

In these measurements, however, not only the excitation but also the measurement is selective: the different kinetics observed on the short-wavelength side and the long-wavelength side of the absorption band might be specific for the pigments measured there, and independent of the pigments initially excited, if the distribution of the excited state over the various pigments in

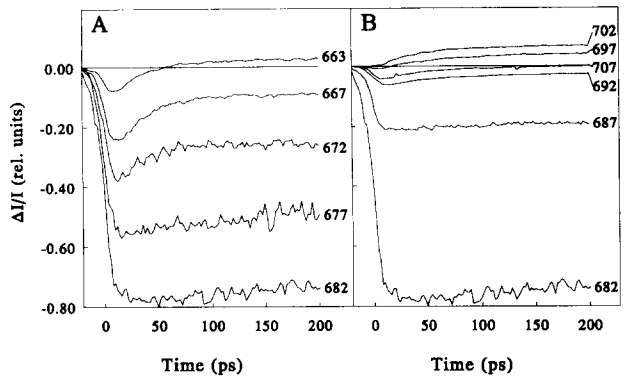

Fig. 2. Absorbance changes of the PS II RC induced by selective excitation of accessory Chl at 662 nm and probing in the accessory Chl (A) and P680 (B) absorbance region. The average excitation energy was 2.9 mW.

the reaction center is thermally equilibrated in 3 ps or less. To investigate this point, two-color pump-probe experiments were carried out. Although the 3 ps component is much faster than the 18 ps response function of the apparatus as a result of jitter between the two dye lasers, the difference between a 3 ps and a 30 ps absorbance increase should still be obvious.

Fig. 2 shows absorbance changes at a series of wavelengths induced by selective excitation of accessory Chl at 662 nm. The 30 ps absorbance increase on the short-wavelength side is expectedly similar to that observed in the one-color measurements of Fig. 1A and is clearly present also at much longer wavelengths, around 700 nm, where it may be attributed to the disappearance of stimulated emission from excited Chl and appearance of $P680^+Pheo^-$ absorbance. At wavelengths from 677 nm upwards a small phase of about 120 ps was observed, but no evidence for a 3 ps phase was found at any wavelength.

Fig. 3 shows corresponding data obtained with selective excitation of P680 at 687 nm. Although nearly hidden by the convolution with the instrument response function, the 3 ps component is clearly present on the long-wavelength side. On the short-wavelength side, the initial bleaching may have a rise time of 3 ps, as would be expected if the 3 ps component reflects charge separation from excited P680, but this is hard to prove because also the point of zero delay between pump and probe pulse is a fit variable. At most wavelengths a small, very slow absorbance increase is observed. At no wavelength could a significant 30 ps phase be detected.

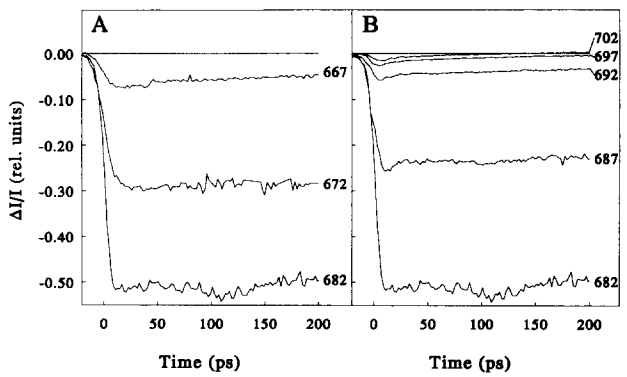

Fig. 3. Absorbance changes of the PS II RC induced by selective excitation of P680 at 687 nm and probing in the accessory Chl (A) and P680 (B) absorbance region. The average excitation energy was 5.4 mW.

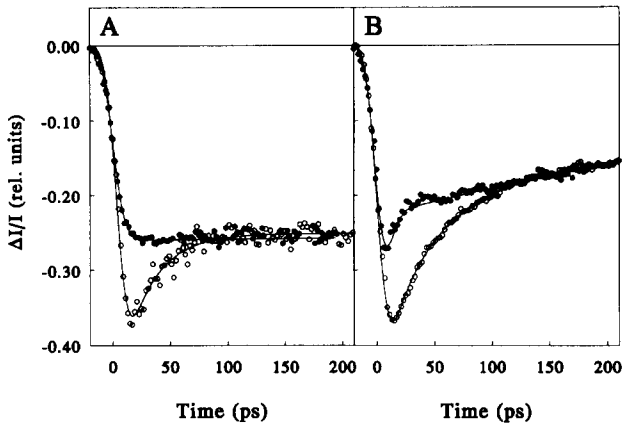

Fig. 4. Absorbance changes at 672 nm (A) and 692 nm (B) upon selective excitation of accessory Chl at 662 nm (open circles) or P680 at 687 nm (solid circles). The absorbance changes at each probing wavelength have been normalized to the same absorbed excitation energy. Experimental conditions are the same as in Fig. 2, 662 nm excitation, and Fig. 3, 687 nm excitation. The lines represent the best fits to the experimental data. The time constants obtained from the fits to the data are (A) 26 ps and > 15 ns for excitation at 662 nm and 687 nm, respectively, and (B) 28 and 110 ps for excitation at 662 nm, and 3 ps and 0.6 ns for excitation at 687 nm.

The conclusion from Figs. 2 and 3 must be that the kinetics depend not only on the wavelength of measurement but also on the pigments initially excited; equilibration of the excited state between those pigments is ruled out. Fig. 4 illustrates that the differences between the four different kinetic components mentioned are significant. It shows measurements at 672 nm (A) and 692 nm (B), normalized to similar amplitudes, upon excitation of accessory Chl (open circles) and of P680 (solid circles), normalized to the same amount of absorbed energy. At 672 nm (Fig. 4A) the characteristic 25–30 ps phase observed upon excitation of accessory Chl (open circles) was absent when P680 was excited (solid circles). The absorbance increase at 692 nm following an instantaneous bleaching upon excitation of P680 (Fig. 4B, solid circles) is obviously at least biphasic and can be fitted within the noise by a bi-exponential function with time constants of 3 ps and 0.6 ns. The value of the latter time constant is a lower limit because of the time window used for the fit. The relative amplitudes of these two components were equal to those obtained from the fit to the one-color 692 nm

data of Fig. 1B, indicating that the different shape of the 692 nm trace in Fig 4B, solid circles, is indeed caused only by the convolution with the instrument response function. The absorbance increase at 692 nm after excitation of accessory Chl (Fig. 4B, open circles) contains at least two components with lifetimes of 28 ps and 110 ps, while a third component with a lifetime of ~ 0.6 ns may slightly improve the fit. Excitation on the short-wavelength side induced the 30 ps phase and no 3 ps phase, and yet, after normalization to the amount of absorbed energy the total amount of $P680^+Pheo^-$ formed as indicated by the absorption differences remaining after 200 ps was within 5% the same as with long-wavelength excitation. The amplitude of the 30 ps phase measured at 672 nm is ~ 20% of the maximum bleaching at 682 nm (disappearance of Chl and Pheo ground states). This is 4-times more than the uncertainty in the yield of $P680^+Pheo^-$ formation upon selective excitation, indicating that the 30 ps phase has to contribute to the charge separation, contrary to suggestions in Refs. 24 and 25.

Since a rapid equilibration of the excited state with the short-wavelength pigments is ruled out, absorbance changes after long-wavelength excitation should reflect the transition from excited long-wavelength pigments, P680* and Pheo*, to the charge-separated state, $P680^+Pheo^-$. The ground-state absorbance of these pigments is not recovered and the only likely origin of the large 3 ps absorbance increase around 687 nm is the disappearance of their stimulated emission.

Short-wavelength excitation produces an initially different state, presumably excited accessory Chl, which takes 10-times longer to generate the same charge-separated state, with the same quantum yield. We conclude therefore that the excited short-wavelength pigments take 30 ps to transfer their energy to the long-wavelength pigments. The reverse transfer plays no role because it must be even slower and cannot compete with the 3 ps charge separation.

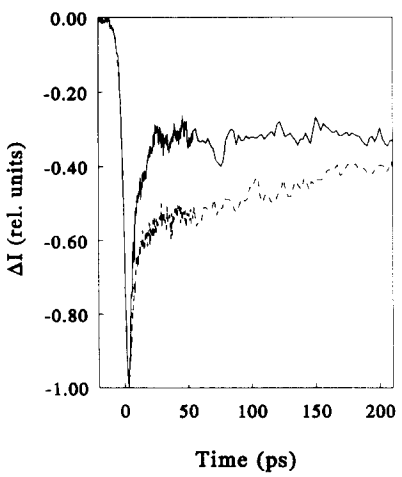

Fig. 5. Absorbance changes of PS II RCs under anaerobic (line) and aerobic (dashed line) conditions at 687 nm upon excitation at the same wavelength. The measurements were normalized on their initial amplitude.

All measurements described above were carried out under anaerobic conditions. Fig. 5 shows the 687 nm data of Fig. 1B together with an identical measurement under aerobic conditions. From these measurements it is clear that the presence of oxygen dramatically changes the kinetics, resulting in a pronounced 120 ps component, at the expense of the 3 ps component. An increase of the 120 ps component under aerobic conditions was observed also at other wavelengths (data not shown). Damage to the RC seems to be the most likely explanation for the prominent appearance of this component in the presence of oxygen, but its molecular origin is not yet clear.

4. Discussion

Upon excitation of isolated PS II reaction centers, an initial absorbance decrease was observed at all wavelengths in the Q_Y-absorption region. In the time range of 1 to 200 ps after excitation this was followed by an increase which revealed time constants of 3, 30 and 120 ps and 0.6 ns. The presence and amplitude of each of these phases was dependent not only on the wavelength of measurement, but also on the wavelength of excitation: excitation on the short-wavelength side of the absorption band induced the 30 and 120 ps phases, excitation on the long-wavelength side induced the 3 ps and 0.6 ns phases.

4.1. The 0.6 ns component

The 0.6 ns phase was observed as an increase on the long-wavelength side of the Q_Y absorption region, suggesting that it reflects the disappearance of stimulated emission from an excited state. Although its presence after short-wavelength excitation cannot be excluded, it seems to be larger upon long-wavelength excitation inducing the same amount of charge separation. This suggests that it is not due to a relaxation or recombination of the radical pair, but to an isolated, long-wavelength pigment. Its amplitude, however, is too small to account for one of the reaction center pigments. An impurity or small fraction of damaged centers in the preparation might be responsible for the 0.6 ns phase.

4.2. The 120 ps component

The 120 ps component was only present upon excitation at the short-wavelength side with detection at longer wavelengths. Its relative contribution was en-

hanced in the presence of oxygen and under these conditions it was also observed upon long-wavelength excitation, concomitant with a decreased amplitude of the 3 ps component. Although the nature of the process responsible for the 120 ps lifetime is not clear, we suspect that it takes place in a relatively small fraction of (partially) damaged reaction centers which do not exhibit normal energy transfer and charge separation kinetics.

4.3. The 30 ps component

The results show that the 30 ps component is due to energy transfer from accessory Chl to P680. This excludes a more rapid equilibration of the excited state between accessory Chl and P680, as postulated in Refs. 23, 31 and 32. Our assignment of the 30 ps component is in agreement with the conclusions drawn from time-resolved fluorescence measurements at 277 K [26,27], but not with the interpretation of a component with a comparable lifetime (21 ps) given in Ref. 30. At low temperature the energy transfer may be different from that at room temperature [13,28].

The spectral distribution of the initial bleaching on excitation of the accessory Chl (Fig. 2) is strongly asymmetrical and has a maximum near 680 nm, probably due to stimulated emission. From 677 to 687 nm it is nearly the same as the difference spectrum of radical pair formation. This observation suggests that the emission spectrum of the accessory Chls is red-shifted by about 10 nm, closely overlapping the absorption spectrum of P680, and that the absorption spectrum of their excited state largely compensates the bleaching of their ground state absorption spectrum on the short-wavelength side. Due to similar electronic configuration, the excited state absorbance of Chl may be similar to the absorption spectrum of the π-anion radical in vitro [35], which has a pronounced maximum on the short wavelength side of the ground state Q_Y transition (but see also [36]). The fact that the total bleaching in the 660–690 nm range decreases only little with 30 ps energy transfer and charge separation is consistent with this interpretation. The integrated oscillator strength initially decreases by twice that of one pigment (bleaching of the ground state and appearance of stimulated emission) and increases by the absorbance of the excited state. After charge separation the absorbance of two pigments, one Pheo and one P680 Chl, is replaced by the absorbance of one reduced Pheo and one oxidized Chl. Hence if the absorbance of reduced Pheo resembles that of excited Chl, the net change in integrated oscillator strength due to energy transfer and charge separation should in first approximation be an absorbance increase amounting to the absorbance of one oxidized Chl, which is probably similar to or somewhat less than that of a reduced pigment [37].

4.4. The 3 ps component

The 3 ps component was assigned to charge separation, because its large amplitude around 685 nm most probably represents disappearance of stimulated emission from P680 upon formation of the charge-separated state (confirming the conclusions in [13,23–28]). Although a different interpretation was given by Durrant et al. [29], our conclusion is not necessarily in contradiction with their data. It seems likely that long-wavelength excitation not only hits P680 but also pheophytin. We do not observe (at room temperature) a slow component that might be due to energy transfer from Pheo to P680, and suggest that between Pheo and P680 a rapid equilibration of the excited state does take place.

On excitation of P680 charge separation occurs within the time resolution of Fig. 3, but the one-color experiments of Fig. 1 show that the initial bleaching in the 680–690 nm region is much larger than that after charge separation, probably due to stimulated emission from excited P680 (and Pheo). The opposite may be true at shorter wavelengths, although our data cannot prove that, but the integrated absorbance over the whole spectral range probably increases by at least the same amount as in the case of accessory Chl excitation. This is expected, because the interpretation of the net increase discussed above should not depend on which pigment is excited initially.

4.5. Sub-picosecond processes

Our conclusion that equilibration of the excited state between the short-wavelength and the long-wavelength pigments would take more than 30 ps necessitates a different explanation for the sub-ps absorbance changes reported by Durrant et al. [31]. Durrant et al. [31] have reported that excitation at 665 nm leads to a shift of the maximum bleaching from 670 to 677 nm with a time constant of 0.1 ps. Our results imply that this cannot be attributed to energy transfer from accessory Chl to P680. It may be due to relaxation processes as reported by McCauley et al. [38]. Excitation of PS II RC at the short-wavelength side of the Q_Y-absorption band results in excited accessory Chl with excess vibrational energy, which is expected to relax to a Boltzmann equilibrium on a sub-ps timescale. This will cause a blue-shift of the excited state absorption and a red-shift of the emission spectrum, resulting in a net red-shift of the initial bleaching. Also a rapid equilibration of the excited state between spectrally different accessory Chl molecules could cause a red-shift, both of the bleached ground state absorbance and of the stimulated emission.

Upon long-wavelength excitation, Durrant et al. observed a 0.1 ps decrease of the initial bleaching by

about a factor of two [31]. This can also be attributed to relaxation of the initially excited state. Our results show that the total bleaching remaining after this process involves at least the oscillator strength of one molecule, so the initial bleaching observed by Durrant et al. must involve at least two. This can be explained if P680 is a dimer with nearly (anti) parallel Q_Y-transition moments of the constituent monomers. All transition probability would be in one of the two exciton bands and the initial bleaching and stimulated emission would be halved with the coherent lifetime of the dimer excited states, which is determined by the correlation-time of fluctuations in the environment [39]. Recent experiments have shown that this time constant is of the order of 0.1 ps [40]. Given that the dimer splitting is relatively small, fluctuations in the environment will redistribute the initial excitation over the two exciton states. A similar redistribution of oscillator strength may also occur upon excitation of weakly interacting accessory Chl. This interpretation would predict that the 0.1 ps component is strongly temperature dependent.

4.6. Structure of P680

Our conclusion that P680 is a dimer with nearly (anti) parallel Q_Y-transition moments of the constituent monomers is consistent with the LD-ADMR measurements on P680 by Van der Vos et al. [14]. It was ruled out by these authors because Kwa et al. [41] found a substantial angle between the absorption dipoles around 690 nm (at low temperature) and the fluorescence emitting dipoles, but this would also rule out monomeric P680, the only alternative allowed by the data of Van der Vos et al. [14]. The observation by Kwa et al. [41] may be explained by energy transfer between P680 and another long-wavelength pigment. The presence of such a pigment is also indicated by a positive LD band at 681 nm. As pointed out in [42], this band does not change upon photoaccumulation of reduced Pheo, whereas both the 680 nm absorbance and the CD in this region are shifted about 2 nm to shorter wavelengths. The data of Van der Vos et al. [14] and Van Mieghem et al. [43] together indicate that the angle between P680 Q_Y-transition moment and the normal to the membrane plane is close to the magic angle, at which no LD is observed. The pigment responsible for the positive LD at 681 nm could well be the 'inactive' Pheo [14]. Since the 'active' Pheo has a negative LD [44] this would not allow C_2-symmetry around an axis perpendicular to the membrane.

The assignment of the conservative CD signal around 680 nm to the P680 dimer [42] is not inconsistent with the notion that nearly all transition probability is associated with one of the two exciton bands, but the width of the signal does suggest that the exciton splitting is very small. By comparison with the reaction center of purple bacteria we estimate that such a small exciton splitting would require a more than twofold larger distance between the magnesium atoms of the special pair, unless the angle between the Q_Y-transition moments (not the molecular Z-axes as proposed in [45]) and their connecting axis is close to the magic angle. The latter option seems more likely. Even so the structure is notably different from that in purple bacteria, because the C_2 symmetry is broken or the symmetry axis is tilted substantially from the normal to the membrane.

4.7. Position of accessory Chls

The 30 ps time required for energy transfer from accessory Chl to P680 implies that the interaction between these pigments is very weak. Therefore [46], the center-to-center distance between the donor and acceptor molecules then may be calculated from the Förster equation for the rate constant of energy transfer [47]:

$$k_{DA} = 8.8 \cdot 10^{17} \frac{\kappa^2}{n^4 R_{DA}^6 \tau_D^F} \int F_D(\nu) \epsilon_A(\nu) \nu^{-4} \, d\nu \qquad (1)$$

In Eq. (1), κ is an orientation factor depending on the relative orientation of the donor and acceptor transition moments, n is the refractive index of the medium, R_{DA} is the center-to-center distance between the donor and acceptor molecules, τ_D^F is the fluorescence lifetime of the donor molecule and ν is the frequency. The so-called overlap integral in Eq. (1) contains the normalized fluorescence emission spectrum of the donor $F_D(\nu)$ and the molar extinction spectrum of the acceptor $\epsilon_A(\nu)$. For the overlap integral a maximum value was calculated under the assumption that the fluorescence emission spectrum of accessory Chl and the absorption spectrum of P680 have a Gaussian shape and have a maximum overlap, with a maximum extinction coefficient of P680 of 150 $mM^{-1} cm^{-1}$. Furthermore, ν was set constant to $4.4 \cdot 10^{14}$ Hz corresponding to a wavelength of 680 nm. Averaging over all possible orientations results in $\kappa^2 = 2/3$, while we took $n = 1.4$ and $\tau_D^F = 15$ ns. For an energy transfer time of 30 ps we now calculate a center-to-center distance between pigments of 30 Å. This may actually be a weighted average of several pairwise energy transfers from each of the 4 accessory Chls to P680/Pheo, in which case the average distance may be somewhat larger than 30 Å. On the other hand the distance of the nearest accessory Chl would be smaller, but even in the unlikely case that all 4 accessory Chls would constitute a single rapidly communicating pool and only one is responsible for the 30 ps transfer, the distance of that Chl would still be larger than 20 Å. 30 Å is a large

distance with respect to the size of the RC protein, but not unreasonable because an edge-to-edge distance of more than 15 Å may be needed to avoid oxidation of accessory Chl by $P680^+$ during its several hundred ns lifetime [48]. Likely binding sites for accessory Chls would be conserved histidines, glutamines or asparagines in the membrane-spanning α-helices of the D1 and D2 proteins. Excluding the histidines in helices IV and V which align with those binding the special pair and the non-heme iron in *Rhodopseudomonas viridis*, and excluding glutamine 165 in helix III which would be too close to tyrosines Z and D, this leaves histidines 118 in the helices II of both D1 and D2, and histidine 62 in helix I of D2 [49]. In *Rps. viridis* the distance between the center of the special pair and the sulfur atom in L cys 92, which aligns with D1 his 118, is 30 Å. Accessory Chl(s) on D1 probably do not communicate with those on D2 and yet we find monophasic energy transfer kinetics to P680/Pheo. This suggests that C_2-symmetry is maintained with respect to the distance between the long-wavelength pigments, P680 and Pheos, and the nearest accessory Chl on either side, with a central position of P680 and D1 and D2 histidines 118 as the positions of the nearest accessory Chls.

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