MiniReview

New and unexpected routes for ultrafast electron transfer in photosynthetic reaction centers

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Received 19 May 1999

Abstract In photosynthetic reaction centers, the excitation with light leads to the formation of a charge separated state across the photosynthetic membrane. For the reaction center of purple non-sulphur bacteria, it was previously generally assumed that this primary charge separation could only start with the excitation of the so-called special pair of bacteriochlorophyll molecules located in the heart of the RC. However, recently new and ultrafast pathways of charge separation have been discovered in the bacterial RC that are driven directly by the excited state of the accessory monomeric bacteriochlorophyll present in the active branch of cofactors. These results demonstrate that the route for energy conversion in photosynthesis can be much more flexible than previously thought. We suggest that the existence of multiple charge separation routes is particularly relevant for the mechanism of charge separation in the photosystem II reaction center of higher plants.

Key words: Photosynthetic reaction center; Primary charge separation; Biological electron transfer; Photosystem II

1. Introduction

Photosynthesis is the process by which plants, algae and photosynthetic bacteria convert light energy into electrochemical energy, that can be used to drive all relevant cellular processes. The biological machinery that accomplishes this energy conversion process is the photosynthetic reaction center (RC), a transmembrane pigment-protein complex that performs a light driven charge separation reaction across the photosynthetic membrane. In oxygenic photosynthesis, a complex electron transport chain connects PS-II with PS-I and the electron transfer is coupled to the transfer of protons across the membrane. Photosynthetic bacteria contain one type of RC that is either related to the PS-I type in green bacteria and heliobacteria or related to the PS-II type in purple bacteria.

For the photosynthetic RCs of purple bacteria high resolution (2.2 Å) three-dimensional X-ray structures have been resolved for the RCs of *Rhodopseudomonas* (*Rps.*) *viridis* and *Rhodobacter* (*Rb.*) *sphaeroides* [1,2]. The protein of the RC is embedded in the bacterial cytoplasmic membrane and is composed of three subunits, H, L and M. The L and M subunits each have five transmembrane helices which encase the cofactors that participate in the light driven electron transfer reaction and are related by an axis of approximate two-fold symmetry, as is depicted for the cofactors in Fig. 1A. A bacteriochlorophyll (BChl) dimer (P) lies close to the periplasmic face of the protein. Two monomeric BChls (B_A and B_B), two bacteriopheophytins (H_A and H_B) and two ubiquinones (Q_A and Q_B) are arranged in two approximately symmetric branches that span the membrane. Thanks to a multitude of spectroscopic studies using advanced molecular and biophysical techniques, detailed information has become available about the primary charge separation reactions [3–5]. It is by now very well established that excitation of the BChl dimer P, either directly or by excitation energy transfer from the antenna, results in the excited state of P (P*), which at room temperature decays in about 3–4 ps. Consensus is emerging that the first formed radical pair is the state $P^+B_A^-$, which within 0.9–1.5 ps decays into $P^+H_A^-$. The $P^+H_A^-$ state on its turn lives for about 200 ps to form $P^+Q_A^-$. Electron transfer from Q_A^- to Q_B takes a few hundreds μs. Given the symmetric structure of the RC, it is remarkable that the primary charge separation occurs almost exclusively along the A branch of cofactors most closely associated with the L subunit (B_A, H_A, Q_A). Most likely the energetics of the intermediate state of $P^+B_A^-$ versus $P^+B_B^-$ plays a crucial role for this directional specificity.

Based upon a 4 Å resolution of PS-I [6] and a low resolution structure of PS-II [7] it has become clear that the two-fold symmetrical arrangement of the cofactors, whereby a dimer of (B)Chl molecules is flanked by four 'monomeric' chlorin cofactors, is a general motif that is also characteristic for the electron transfer chain of the RCs of other photosynthetic species (see [8] for a recent review). On the basis of these structures one might predict that the nature and mechanism of the early electron transfer events in photosynthetic RCs display strong similarities. However, the spectroscopic properties of plant RCs, in which all chlorin factors absorb in the same narrow spectral region, strongly differ from those of purple bacterial RCs for which the cofactors absorb at clearly distinguishable wavelengths (see Fig. 1B for a comparison of a low temperature absorbance spectrum of the RCs of PS-II and *Rb. sphaeroides*). In particular, the long wavelength transition that in the RC of photosynthetic purple bacteria is ascribed to the lowest excitonic state of the special pair, can not be observed in the plant RC. As a result thermal equilibration in plant RCs does not result in the localization of the excitation energy on a special pair. This is in contrast to the bacterial

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Abbreviations: BChl, bacteriochlorophyll; BPhe, bacteriopheophytin; D, heterodimeric primary electron donor; P, homodimeric primary electron donor; Q, quinone; *Rb*, *Rhodobacter*; RC, reaction center; WT, wild-type; PS, photosystem

PII: S0014-5793(99)00810-8

RCs in which the excitation energy will be funnelled to the lowest optical transition of the BChl dimer.

In this minireview we discuss new routes of charge separation that have recently been discovered in mutant and WT RCs of *Rb. sphaeroides* and that have challenged the unwritten dogma that ultrafast electron transfer in the RC absolutely requires the presence of a 'special pair' of a (B)Chl dimer located in the heart of the RC. We propose that these new routes of charge separation are of particular interest to understand the mechanism of charge separation in RCs for which the lowest excited state is not supposed to be localized on a dimer of chlorin cofactors.

2. New routes for primary charge separation in bacterial RCs not involving P*

From a multitude of experiments with sub-picosecond time resolution, it was generally concluded that upon excitation of any of the monomeric bacteriochlorin cofactors, excitation energy is rapidly funnelled downhill to the BChl dimer on a time-scale of a few hundreds fs (see [9] for a recent review). However, a quantitative estimation of the efficiency of energy transfer is not performed in these experiments. In WT RCs a measurement of the fluorescence excitation spectrum is complicated due to the short lifetime of the P* state, which causes that the amount of prompt fluorescence is very low. Therefore, a large fraction of the integrated fluorescence may arise from slow recombination fluorescence that originates from the dynamic equilibrium between P* and $P^+H_A^-$ [4,10]. By site-directed mutagenesis RCs have been obtained, in which the primary charge separation driven by P* is dramatically slowed down. One of those RCs is the YM210W RC in which the tyrosine residue at the M210 position is changed into a bulky tryptophan residue (see [11] for the X-ray structure of this RC). Because of the long P* lifetime (~400 ps at 77 K), the amount of prompt fluorescence at cryogenic temperature is raised by a factor of ›200 compared to the WT RC, which makes this YM210W RC a perfect candidate for a measurement of the energy transfer efficiency from the accessory RC pigments to P via a steady state fluorescence excitation spectrum [12]. In total contrast to our original expectations it appeared from these measurements that most of the excitation energy residing on B_A and H_A is not transferred to P to generate the fluorescent state P*. On the other hand, the action spectrum of $P^+Q_A^-$ formation in this mutant showed that the excitations that were missing in the P* excitation spectrum were in fact used to perform charge separation. These observations led to the unavoidable conclusion that upon excitation of B_A and H_A not only energy transfer to P may occur, but that in addition an ultrafast electron transfer process driven by H_A* and/or B_A* efficiently competes with this energy transfer. Similar results were obtained for the YM210F, YM210L, one of the so-called high potential mutant RCs, LM160H+FM197H [13], and the HM202L RC, in which the excitonically coupled BChl homodimer is converted to a BChl: BPhe heterodimer (also called D) [14]. In this heterodimer RC, B excitation was in fact about two times more efficient in generating charge separation than D excitation.

Subsequent sub-ps absorbance measurements demonstrated unequivocally the existence of at least two new ultrafast pathways of primary charge separation, besides the well established route driven by P*. These new paths start from

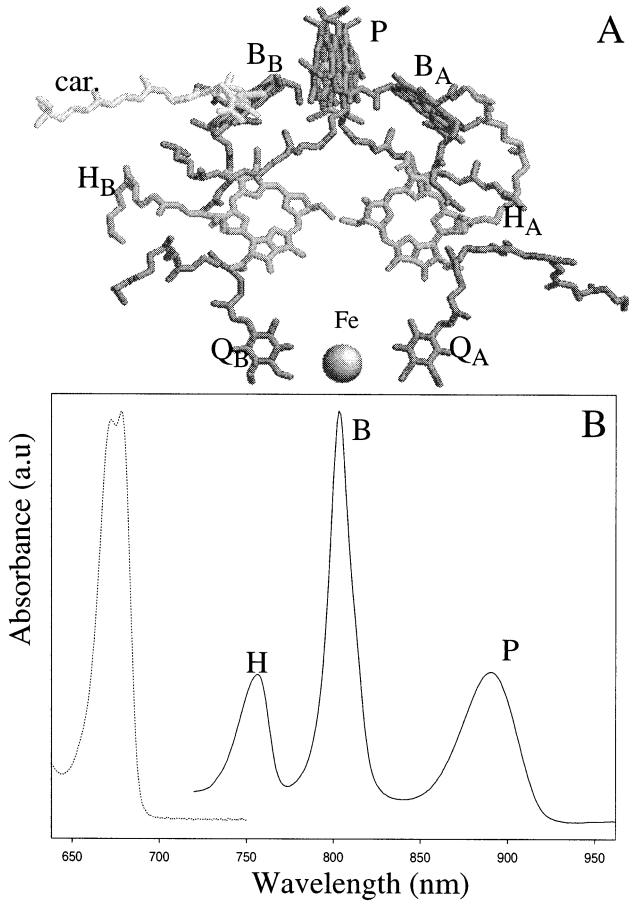

Fig. 1. A: Schematic of the arrangement of the cofactors present in the *Rb. sphaeroides* RC. Four BChl molecules (B_A, B_B and two which form an excitonically coupled pair (P)), two BPhe molecules (H_A and H_B) and two ubiquinones (Q_A and Q_B) are arranged in two near symmetric branches that span the membrane. The remaining cofactors are a carotenoid molecule (car) and a non-heme Fe atom. B: 77 K absorption spectrum of the Q_y region of the membrane bound RC of *Rb. sphaeroides* (solid) [16] and of the isolated PS-II RC from spinach (dotted) [31]. In the Q_y region of *Rb. sphaeroides* three separated absorbance bands of the B, H and P cofactors can be distinghuished between 700 and 950 nm, as is indicated in the figure. The cofactors of PS-II all absorb in the same narrow spectral region between 650 and 700 nm.

the excited state of B_A (B_A*) and were shown to involve the primary reactions $B_A^* \rightarrow B_A^+H_A^- \rightarrow P^+H_A^-$ and $B_A^* \rightarrow P^+B_A^- \rightarrow P^+H_A^-$ [14–16]. For a detailed description of the kinetic and spectral properties of these alternative charge separation paths we refer to these articles. In this minireview we will only illustrate the sometimes dramatic appearance of the alternative charge separation in the YM210W, the WT and the HM202L heterodimer RC in transient absorbance measurements, as depicted in Fig. 2. The operation of the electron transfer routes strongly differs in these three RC systems and we will discuss how specific interactions between the cofactors may influence the 'new' charge separation processes driven by B* and the 'regular' pathway of charge separation from P*. The reaction schemes and energetics for these three systems are summarized in Fig. 3A–C.

In YM210W RCs at 77 K P* driven charge separation is very much slowed down and proceeds with ~400 ps lifetime and results in a 60–70% quantum yield (QY) of $P^+Q_A^-$ for-

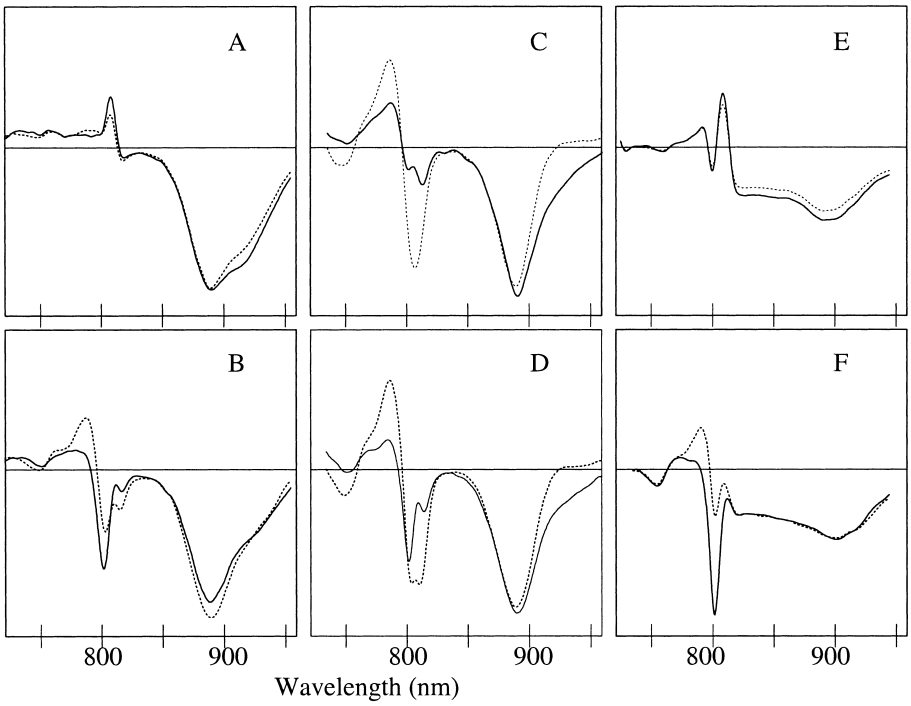

Fig. 2. Transient absorbance difference spectra obtained at 77 K after 1 ps (solid) and 10 ps (dotted) after 796–799 nm excitation and 880 nm excitation of the YM210W (A, B), WT (C, D) and HM202L heterodimer RC (E, F). The spectra were reconstructed from a global analysis fit as described previously [14–16]. A: YM210W RC 880 nm excitation. The spectra recorded at 1 ps and 10 ps after direct excitation of P at 880 nm (Fig. 2A) show only contributions from P*. The positive signal at 808 nm is also characteristic for P*. The decrease in the amount of P* stimulated emission between 1 and 10 ps is interpreted as a spectral evolution of P* that is not associated with the charge separation process. B: YM210W RC 799 nm excitation. The transient absorbance spectra obtained 1 ps after 799 nm excitation show P* stimulated emission, P ground state bleaching, a bleach of H absorbance (superimposed on P* excited state absorbance) and a strong bleach of B absorbance. The amount of P* stimulated emission is however less than with the 880 nm excitation experiment. The bleach of B and H absorbance and the limited amount of P* stimulated emission is interpreted as a combination $P^+B_A^-$, P* and $B_A^+H_A^-$. At 10 ps a spectrum with an electrochromic bandshift of the B absorbance band is present, indicating that $P^+B_A^-$ and $B_A^+H_A^-$ have been converted to $P^+H_A^-$. Note that the amount of P* stimulated emission remains equal between 1 and 10 ps, which indicates that P* is not involved in these changes. The amount of P ground state absorbance bleaching does increase, which is indicative for the transition $B_A^+H_A^- \rightarrow P^+H_A^-$. C: WT RC 880 nm excitation. The spectrum recorded at 10 ps resembles the $P^+H_A^-$ difference spectrum. At 1 ps after excitation about 50% of P* has been converted to $P^+H_A^-$ and the spectrum represents a mixture of P* and $P^+H_A^-$. D: WT RC 796 nm excitation. The spectrum recorded at 10 ps resembles the $P^+H_A^-$ difference spectrum and is similar to the spectrum recorded 10 ps after 880 nm excitation. The spectrum recorded at 1 ps shows besides the characteristics of P* and $P^+H_A^-$ an additional bleach of B absorbance which is interpreted as a contribution of the $P^+B_A^-$ intermediate that was formed directly from B*. E: HM202L heterodimer RC 880 nm excitation. The difference spectra recorded at 1 and 10 ps are similar, except for the amplitude of the 10 ps spectrum which has decreased by 15% in amplitude. The spectra are attributed to D*. The characteristic features in the region between 780 and 820 nm are interpreted as electrochromic bandshifts of the B absorbance bands, which are induced by the pronounced charge transfer character of the D* state. F: HM202L heterodimer RC 798 nm excitation. The difference spectrum recorded at 1 ps after B excitation is attributed to a combination of D* and $B_A^+H_A^-$ which have both been formed from B* on a sub-picosecond time-scale. At 10 ps after 798 nm excitation the strong bleach of the B absorbance band has evolved into an electrochromic bandshift, whereas the bleach of D and H absorbance remains resembling the reaction $B_A^+H_A^- \rightarrow D^+H_A^-$. Some of the formed D* has decayed to the ground state between 1 and 10 ps (see E), therefore the delayed bleach of D caused by extra D^+ could be cancelled by the decay of D* to ground state.

mation, from which time constants of 1.3 ns for the decay of P* to the ground state and of $\sim$570 ps for P* driven charge separation can be calculated. The slow P* driven charge separation in this mutant is largely caused by a $\sim$50 meV increase of the energy level of the $P^+B_A^-$ intermediate state, which puts the energy levels of P* and $P^+B_A^-$ at approximately iso-energetic position [5,22]. Therefore, the spectra recorded at 1 and 10 ps after 880 nm (P) excitation only show contributions of P* (Fig. 2A). The P* driven charge separation in the YM210W RCs at 77 K proceeds either via a sequential mechanism, whereby $P^+B_A^-$ or $B_A^+H_A^-$ is involved as a true intermediate, or via a superexchange mechanism, whereby the electron is transferred directly from P* to $P^+H_A^-$ and the electronic coupling could be enhanced via mixing with the $P^+B_A^-$ or $B_A^+H_A^-$ states [41,42]. These two possibilities are indicated by the dotted arrows in Fig. 3A. Upon excitation of B at 799 nm (Fig. 2B) the spectral evolution is completely different from that following P excitation and it is very clear that not only energy transfer from B* to P occurs in complete agreement with the steady state fluorescence excitation spectrum [15]. From this experiment we conclude that from B* within a few hundreds fs a mixture of three states is formed: P*, $P^+B_A^-$ and $B_A^+H_A^-$ (see Fig. 2B, solid). The typical electrochromic bandshift of the B absorbance band, that is observed in the spectrum recorded at 10 ps, shows that by that time the $P^+B_A^-$ and $B_A^+H_A$ formed from B* have converted to $P^+H_A^-$, while the fraction of P* still remains (Fig. 2, dotted). In Fig. 3A we have summarized the energy level diagram for the YM210W RC and included the estimated time constants.

In WT RCs excitation of P at 77 K leads to the formation of the radical pair $P^+H_A^-$ within 1–2 ps. Consequently, the spectrum recorded 10 ps after 880 nm excitation (Fig. 2C,

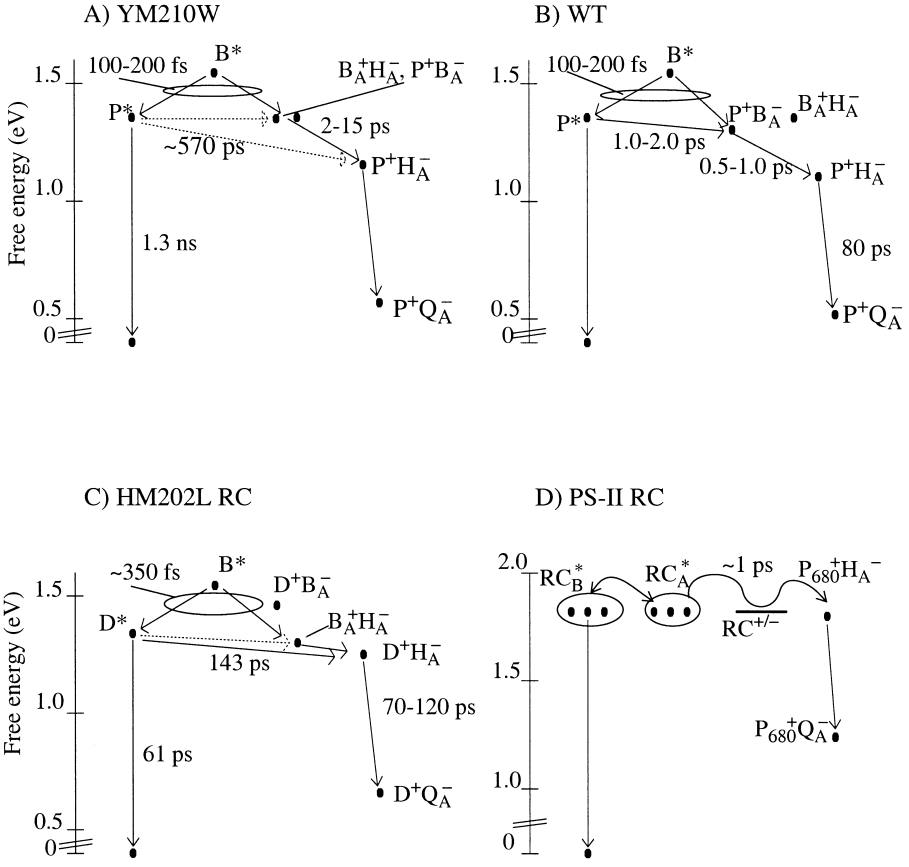

Fig. 3. Schematic of the electron transfer reactions at 77 K driven by P* and B* and the energy level of the charge separated states in the YM210W (A), WT (B) and HM202L heterodimer (C) RCs of *Rb. sphaeroides* and the PS-II RC of higher plants (D). The free energy levels of $P^+B_A^-$, $P^+H_A^-$ and $P^+Q_A^-$ in the WT RC are described in [4,5]. The energy levels of these states in the YM210W and HM202L RC were estimated according to the changes in the redox potential of the P/P^+ and D/D^+ redox couple, respectively, as compared with the WT RC. The energy levels of $B_A^+H_A^-$ and P* are supposed to be iso-energetic, based on theoretical calculation using the crystal structure [41,42].

solid) represents that of $P^+H_A^-$. The spectrum recorded 10 ps after excitation of B (Fig. 2C, dotted) is very similar to that obtained after 880 nm excitation. Fully consistent with the hypothesis of the existence of multiple ultrafast, electron transfer paths in photosynthetic RCs, we observe that the spectrum at 1 ps after B excitation shows an additional bleach of the B absorbance band (Fig. 2D, dotted), which reflects the contribution of $P^+B_A^-$ formed in less than a few hundreds fs directly from B*. In contrast to the YM210W mutant no evidence is found for the transient formation of the $B_A^+H_A^-$ intermediate in the WT RCs, i.e. in the WT RCs the formation of $P^+H_A^-$ is not associated with an additional P bleach on a slower time-scale and no clear H bleach was observed in the spectrum directly after the disappearance of B* [16]. Possibly, this is related to the fact that $P^+B_A^-$ is lower in energy in WT RCs compared with YM210W RCs, which makes that in the competitive processes occurring from B*, $P^+B_A^-$ and P* are formed faster in WT RCs. Room temperature experiments with 800 nm B band excitation on membrane bound WT RCs with Q_A reduced could also be interpreted with a parallel formation of $P^+B_A^-$ and P* from B* and did not yield indications that $B_A^+H_A^-$ is formed as an intermediate [17]. However, we remark that other groups, who have performed similar experiments on isolated R-26 WT RCs with Q_A reduced have suggested that also $B_A^+H_A^-$ can be formed from H*/B* in WT RCs [18,19] and that possibly also spectral heterogeneity and multi-exponential energy transfer contribute to the variation in spectral evolution that is observed for the different excitation wavelengths [20]. The putative role of $B_A^+H_A^-$ in the alternative charge separation pathways as suggested by these two groups is however based upon observations at different delay times. In the experiments of Vos et al. performed at 10 K after a delay of 4 ps additional bleaches in the B and H region were observed, in particular for 770 nm excitation (compared with P excitation) [19]. At this delay P* has completely converted into $P^+H_A^-$ and since no spectra were recorded at later times it was unclear whether this 770 nm formed $B_A^+H_A^-$ eventually would convert to $P^+H_A^-$ and yield a successful charge separation. In experiments of Lin et al. performed at room temperature transient bleaches of the B and H were observed at short delay times (500 fs and 1 ps) after excitation of the H band [20]. In these experiments, in agreement with our results on the WT RC, there was no significant bleach of H formed directly from B*. Possibly direct excitation of H_A is required for the formation of $B_A^+H_A^-$ in WT RCs, but this remains to be investigated. In all cases there appears to be less P* stimulated emission upon excitation of the monomeric pigments compared with excitation of P. Recently, several groups have attempted to characterize the initial dynamics within the B band. Three pulse photon echo peakshift (3PEPS) experiments performed by Groot et al. [21] using selective detection of the echo demonstrated a remarkable difference between B_B (red) end B_A (blue) detection. The most straightforward interpretation of the observed peak-

shift decay was the ultrafast formation of $P^+B_A^-$ following B_A excitation in full agreement with our work.

In the HM202L heterodimer RC, the excitonically coupled BChl homodimer has been destroyed and is converted into a BChl:BPhe heterodimer (D) [23]. The absorbance spectrum of this heterodimer is positioned at approximately the same position as the P homodimer in WT RCs, but is significantly broadened and the maximum amplitude is reduced by a factor of $\sim$3. The properties of the charge separation driven by D* are significantly changed. These changes appear to originate largely from the 200–300 mV higher redox potential of the BPhe half of the dimer compared with the BChl part and consequently in the oxidized heterodimer, D^+, the positive charge is no longer symmetrically distributed between the two halves of the dimer, but is fully localized on the BChl part [24]. The redox potential of D has been increased by about 140 mV [24] compared to P in WT RCs and accordingly the energy levels of $D^+H_A^-$ and $D^+B_A^-$ probably have increased by the same amount compared to the WT RC (see Fig. 3C). Excitation of D in membrane bound HM202L RCs at 880 nm results in a 43 ps decay of D*, which leads for about 30% to the formation of $D^+Q_A^-$ and for about 70% to a direct decay to the ground state. Consequently, the spectra recorded at 1 and 10 ps after 880 nm excitation (see Fig. 2E) mainly reflect D*. The low quantum yield of charge separation is the result of both a slower intrinsic rate of charge separation, which is related to the increased energy level of the $D^+B_A^-$ and $D^+H_A^-$ intermediates, and a faster rate of internal conversion to the ground state, which is caused by the large charge displacement in the D* excited state [25]. As Fig. 2E demonstrates, excitation at 798 nm results partly in energy transfer from B* to D and partly in a direct charge separation process yielding the state $B_A^+H_A^-$. In contrast to the YM210W and the WT RCs, there is now no direct formation of $D^+B_A^-$ from B*. Possible explanations could be the significant decrease in the energy gap between B* and $D^+B_A^-$ or, alternatively, a stabilization of $B_A^+H_A^-$ by the permanent dipole moment in the ground state of D. At 10 ps the $B_A^+H_A^-$ that was formed directly from B_A* has been converted to $D^+H_A^-$ (see Fig. 2F). Since the intermediate $D^+B_A^-$ probably lies too high in energy to be involved as a true intermediate in the D* charge separation pathway, we suggest that maybe $B_A^+H_A^-$ is also involved in enhancing the coupling between D* and $D^+H_A^-$ or as a true intermediate formed from D* whereby some B* must be mixed with the D* excited state (see Fig. 3C, dotted line).

Finally, we note that although in Fig. 3 the energy transfer and direct charge separation from B* are shown as competitive paths, in reality we do not know. The energy transfer may be the only process following B_B excitation, while for B_A* there may be competition between energy transfer to P and direct charge separation.

A comparison of the results obtained on these different RCs clearly demonstrates the presence of at least three different charge separation routes that all lead to the formation of $P^+H_A^-$. Which route of charge separation is chosen strongly depends on the initial position of the excitation and on the energy levels of the intermediates and the specific interactions in the RC system. We finally remark that in WT bacterial strains, the RC receives most of its excitation energy from the core antennae which are only resonant with P and therefore the P* driven charge separation route is probably the physiologically most relevant process. However, for the plant RCs in which the spectral contribution of the 'special pair' can not be easily distinguished, the true situation may be much more complicated.

3. The photosystems II and I RCs of higher plants

The photosystem II RC of higher plants and algae was for the first time obtained in a purified form by Nanba and Satoh [26]. The main pigment binding protein constituents are the D1 and D2 polypeptides, which were shown to be homologous to the L and M subunits of the bacterial RC (see for instance [27]). A large body of evidence has accumulated to demonstrate that the D1D2 particle of PS-II contains six Chls-*a* and two Phes-*a* [28]. Two Chls-*a* are supposed to be located in the periphery of D1D2, and four Chls-*a* and two Phes-*a* are in the core [28]. Consequently, the pigment arrangement in the PS-II RC and the mechanism of electron transfer involving a 'special pair' were speculated to be very similar in both types of RCs. However, in contrast to the purple bacterial RC, the absorption spectrum of the photosystem II RC does not reveal a clear transition that is separated from the absorption of the other RC chromophores that can be assigned to a special pair. Furthermore, EPR experiments on PS-II membranes [30] and polarized transient absorption experiments on isolated PS-II RCs performed at very low temperature [31], furthermore revealed that the PS-II RC triplet state is localized on a single chromophore that is oriented relative to the membrane normal like the voyeur BChls in the bacterial RC [29]. In vibrational spectroscopic work, localization of the triplet on a single chromophore was detected at low temperature ($T < 80$ K); whereas experiments at higher temperature revealed an equilibration of the triplet over two cofactors [29,32]. From a variety of spectroscopic experiments an upper limit of 140 cm^{-1} was obtained for the excitonic interaction between the chromophores constituting P680 [33,43], which should be compared to 550 cm^{-1} for the special pair in *Rb. sphaeroides* RCs and 950 cm^{-1} for the special pair of *Rps. viridis*. Assuming 'similar' structures for both the bacterial and the plant PS-II RC a simple 'explanation' for the difference in spectral appearance is that in PS-II RC the two halves of the 'special pair' have moved apart thereby weakening the interaction, a feature that seems to be consistent with a detailed modelling study [34] and with the low resolution structural model that was recently proposed [7]. If the structural arrangement of the other chlorin cofactors in the PS-II RC and bacterial RC is maintained then the dipole-dipole couplings between the monomeric Chl molecules and the Phe molecules are of the order of 100 cm^{-1}, which is of similar magnitude as the coupling strength that is experimentally observed for P680. A straightforward calculation taking into account the energetic disorder (inhomogeneous broadening) has shown that depending on the specific realization of the energetic disorder, the excited state in the PS-II RC may be (de-)localized on any combination of (on the average) three pigments [35]. There is no special preference for the excitation to be localized on the pigments that structurally correspond to the 'special pair'. In fact, on the average the excitonic states split into two with one on the average 'localized' on the pigments of the active branch of cofactors and the other state on the inactive branch [36]. These states are denoted RC_A* and RC_B* in Fig. 3D. Both excitoned

states are red shifted compared to 'monomeric' Chl-*a* and have a maximal oscillator strength around 680 nm, but dependent on the specific realization of the disorder one is 'blue shifted' and the other 'red shifted' or the other way around [36]. This multimer model was able to explain a number of basic observations on the PS-II RC. For instance, assuming that the state that is localized on the inactive branch (RC_B*) could not give rise to ultrafast charge separation, the model elegantly explained the existence of a trap state in the PS-II RC that becomes fluorescent upon cooling below 50 K [37] and whose spectrum can be identified by the burning of very narrow holes at 1.2 K as a function of the burning laser frequency [38]. Another specific example is the fact that upon excitation at 694 nm, at the very red edge of the absorption spectrum of PS-II, Phe-*a* seemed to participate in the excited state thus formed [36]. Of course it seems most likely that the P680 cation will be localized on the Chl-*a* molecule (part of the 'special pair') that is near the Tyr Z and thus involved in extracting electrons from the water splitting complex.

From a study of the temperature dependence of charge separation in PS-II RCs it was furthermore concluded that the time constant of charge separation in the PS-II RC increased from 2.6 ps at 20 K to 400 fs at room temperature [39], in contrast to the analogous experiment in the WT RCs of purple bacteria, in which the P* driven separation speeds up with decreasing temperature [40]. An interpretation of this observation is that the initial step of charge separation in the PS-II RC is slightly activated and involves the formation of some kind of charge transfer or radical pair state, which subsequently decays into the $P_{680}^{+}Phe^{-}$ radical pair, a few tens of meV below the state that was originally excited by the laser pulse (P680*) [29]. In view of the observed multiple pathways for charge separation in the bacterial RC it is attractive to propose that also in the PS-II RC on an ultrafast time-scale a variety of charge separated states can be formed from any of the excited states that is (de-)localized over the pigments in the active branch. In Fig. 3D these possible charge separated states are summarized as $RC^{+/-}$. The excitations on the pigments present in the inactive branch (RC_B*) are in equilibrium with those on the active branch (RC_A*), which gives rise to a variety of equilibration times. However, for those RCs in which the exciton state on the inactive branch is the most red the excitation will be trapped and lost for successful charge separation at temperatures below 50 K [37].

4. Concluding remarks

We have demonstrated the existence of multiple ultrafast routes for charge separation in bacterial RCs. These experiments suggests that a 'special' pair is not an absolute requirement for ultrafast electron transfer in RCs. This of course nicely fits to the idea that in plant RCs a special pair can not really be distinguished. Possibly from the initial delocalized excited state a multitude of charge separated states may be formed in the PS-II RC, which on a time-scale of a few ps all lead to successful charge separation. Of course, it seems essential that the final P680 cation will be localized on the Chl-*a* molecule (part of the 'special pair') that is near the Tyr Z and thus involved in extracting electrons from the water splitting complex. One might wonder about the reason for a special pair primary electron donor in the bacterial RC. Since the surrounding LH1 antenna, largely due to favorable pigment-pigment interactions, has shifted to 870 nm, the RC must 'follow' this spectral red shift and one possible speculation could be that this red shifting of the RC absorbance could only be realized by a tightly coupled pair of BChl molecules. For plant RCs this requirement is much less strict and as a consequence the 'special pair' is absent. In fact in the plant case the system may have tried to optimize the energy content of the initial charge separated state, a strategy which would want to avoid strongly coupled dimers.

Acknowledgements: The authors wish to acknowledge the very important contributions of Michael R. Jones, Frank van Mourik and Ivo van Stokkum to all the original work. The stimulating discussions with Jan Dekker, Marie-Louise Groot, David Klug and James Durrant were very helpful. The research shown in this work was financially supported by The Netherlands Organization for Scientific Research (NWO) through the Foundation of Earth and Life Sciences (ALW).

References

[1] Fritzsch, G., Kampmann, L., Kapaun, G. and Michel, H. (1998) Photosynth. Res. 55, 127–132.
[2] Stowell, M.H.B., McPhillips, T.M., Rees, D.C., Soltis, S.M., Abresch, E. and Feher, G. (1997) Science 276, 812–816.
[3] Parson, W.W. (1996) in: D.S. Bendall (Ed.), Protein Electron Transfer, Bios Scientific publishers, Oxford, pp. 125–148.
[4] Woodbury, N.W. and Allen, J.P. (1995) in: R.E. Blankenschip, M.T. Madigan and C.E. Bauer (Eds.), Anoxygenic Photosynthetic Bacteria, Kluwer, Dordrecht, pp. 527–557.
[5] Zinth, W., Huppmann, P., Arlt, T. and Wachtveitl, J. (1998) Philos. Trans. R. Soc. Lond. A 356, 465–476.
[6] Krauß, K., Schubert, W.D., Klukas, O., Fromme, P., Witt, H.T. and Saenger, W. (1996) Nat. Struct. Biol. 3, 965–973.
[7] Rhee, K.H., Morris, E.P., Barber, J. and Kühlbrandt, W. (1998) Nature 396, 283–286.
[8] Allen, J.P. and Williams, J.C. (1998) FEBS Lett. 438, 5–9.
[9] Granakaran, S., Haran, G., Kumble, R. and Hochstrasser, R.M. (1999) in: D.L. Andrews and A.A. Demidov (Eds.), Resonance Energy Transfer, Wiley, Chichester, pp. 308–355.
[10] Müller, M.G., Griebenow, K. and Holzwarth, A.R. (1993) Chem. Phys. Lett. 199, 465–469.
[11] Fyfe, P.K., McAuley-Hecht, K.E., Ridge, J.P., Prince, S.M., Gunter, F., Isaacs, N.W., Cogdell, R.J. and Jones, M.R. (1998) Photosynth. Res. 55, 133–140.
[12] Van Brederode, M.E., Jones, M.R. and van Grondelle, R. (1997) Chem. Phys. Lett. 268, 143–197.
[13] Van Brederode, M.E., Ridge, J.P., vanStokkum, I.H.M., van Mourik, F., Jones, M.R. and van Grondelle, R. (1998) Photosynth. Res. 55, 141–146.
[14] Van Brederode, M.E., van Stokkum, I.H.M., Jones, M.R. and van Grondelle, R. (1999) Biochemistry, in press.
[15] Van Brederode, M.E., Jones, M.R., van Mourik, M.R., van Stokkum, I.H.M. and van Grondelle, R. (1997) Biochemistry 36, 6855–6861.
[16] Van Brederode, M.E., van Mourik, F., van Stokkum, I.H.M., Jones, M.R. and van Grondelle, R. (1999) Proc. Natl. Acad. Sci. USA 96, 2054–2059.
[17] Van Stokkum, I.H.M., Beekman, L.M.P., Jones, M.R., van Brederode, M.E. and van Grondelle, R. (1997) Biochemistry 36, 11360–11368.
[18] Lin, S., Taguchi, A.K.W. and Woodbury, N.W. (1996) J. Phys. Chem. 100, 17067–17078.
[19] Vos, M.H., Breton, J. and Martin, J.-L. (1997) J. Phys. Chem. 101, 9820–9832.
[20] Lin, S., Jackson, J., Taguchi, A.K.W. and Woodbury, N.W. (1998) J. Phys. Chem. 102, 4016–4022.
[21] Groot, M.-L., Yu, J.-Y., Agarwal, R., Norris, J.R. and Fleming, G.R. (1998) J. Phys. Chem. 102, 5923–5931.
[22] Beekman, L.M.P., van Stokkum, I.H.M., Monshouwer, R.,

Rijnders, A.J., McGlynn, P., Visschers, R.W., Jones, M.R. and van Grondelle, R. (1996) J. Phys. Chem. 100, 7256–7268.
[23] Bylina, E.J. and Youvan, D.C. (1988) Proc. Natl. Acad. Sci. USA 85, 7226–7231.
[24] Allen, J.P., Artz, K., Lin, X., Williams, J.C., Ivancich, A., Albouy, D., Mattioli, T.A., Fetsch, A., Kuhn, M. and Lubitz, W. (1996) Biochemistry 35, 6612–6619.
[25] Laporte, L., McDowell, L.M., Kirmaier, C. and Schenck, C.C. (1993) Chem. Phys. 176, 615–629.
[26] Nanba, O. and Satoh, K. (1987) Proc. Natl. Acad. Sci. USA 84, 109–112.
[27] Michel, H. and Deisenhofer, J. (1988) Biochemistry 27, 1–7.
[28] Eijckelhoff, C. and Dekker, J.P. (1995) Biochim. Biophys. Acta 1231, 21–28.
[29] Diner, B.A. and Babcock, G.T. (1996) in: D.R. Ort and C.F. Yocum (Eds.), Oxygenic Photosynthesis: The Light Reactions, Kluwer, Dordrecht, pp. 213–247.
[30] Van Mieghem, F.J.E., Satoh, K. and Rutherford, A.W. (1991) Biochim. Biophys. Acta 1058, 379–385.
[31] Kwa, S.L.S. (1993) Ph.D. thesis, Vrije Universiteit, Amsterdam.
[32] Noguchi, T., Inoue, Y. and Satoh, K. (1993) Biochemistry 32, 7186–7195.
[33] Braun, P., Greenberg, B.M. and Scherz, A. (1990) Biochemistry 29, 10376–10378.
[34] Svensson, B., Etchebest, C., Tuffery, P., van Kan, P.J.M., Smith, J. and Styring, S. (1996) Biochemistry 35, 14486–14502.
[35] Durrant, J.R., Klug, D.R., Kwa, S.L.S., van Grondelle, R., Porter, G. and Dekker, J.P. (1995) Proc. Natl. Acad. Sci. USA 92, 4798–4805.
[36] Merry, S.A.P., Kumazaki, S., Tachibana, Y., Joseph, D.M., Porter, G., Yoshihara, K., Barber, J., Durrant, J.R. and Klug, D.R. (1996) J. Phys. Chem. 100, 10469–10478.
[37] Groot, M.-L., Peterman, E.J.G., van Kan, P.J.M., van Stokkum, I.H.M., Dekker, J.P. and van Grondelle, R. (1994) Biophys. J. 67, 318–330.
[38] Groot, M.-L., Dekker, J.P., van Grondelle, R., den Hartog, F.T. and Völker, S. (1996) J. Phys. Chem. 100, 11488–11495.
[39] Groot, M.-L., van Mourik, F., Eijckelhoff, C., van Stokkum, I.H.M., Dekker, J.P. and van Grondelle, R. (1997) Proc. Natl. Acad. Sci. USA 94, 4389–4394.
[40] Fleming, G.R., Martin, J.-L. and Breton, J. (1988) Nature 333, 190–192.
[41] Creighton, S., Hwang, J.-K., Warshel, A., Parson, W.W. and Norris, J.R. (1988) Biochemistry 37, 774–781.
[42] Scherer, P.O.J. and Fischer, S.F. (1989) Chem. Phys. 115, 151–158.
[43] Kwa, S.L.S., Eijkelhoff, G., Van Grondelle, R. and Dekker, J.P. (1994) J. Phys. Chem. 98, 7702–7711.