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Similarity of primary radical pair recombination in photosystem II and bacterial reaction centers

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We report temperature and magnetic field dependent measurements of the recombination dynamics of the radical pair $P680^+Pheo^-$ in $D_1D_2cytb_{559}$ reaction centers of photosystem II and compare the results to those obtained in bacterial reaction centers. In photosystem II the rate of recombination to the groundstate is found to be slower than in the bacterial reaction centers by a factor of at least 50. This difference arises from the different redox potentials of the pigments of plant and bacterial reaction centers. In contrast, the rate of recombination to the triplet state is similar in all reaction centers, indicating a similar electronic coupling which allows us to conclude upon the structural similarity.

Photosynthesis; Reaction center; Photosystem II; Radical pair; Charge recombination

1. INTRODUCTION

Compared to the bacterial photosynthetic reaction center (RC), the primary processes in photosystem II (PS II) of plants are not well understood yet. Only recently the preparation of stable photoactive RCs of PS II was achieved [1,2] which allow spectroscopic measurements with high resolution. These D_1D_2-preparations contain 4–6 chlorophylls *a* (Chl), 2 pheophytins *a* (Pheo), 1 β-carotene, but no quinones [3]. Electron transfer from the primary donor P680 to a Pheo was reported to occur in a few picoseconds even at low temperatures [4,5], although measurements with better time resolution point to a slightly slower charge separation preceded by energy transfer processes [6]. $P680^+Pheo^-$ decays on the nanosecond timescale [7–9] by recombination via the radical pair mechanism, as can be concluded from the magnetic field dependence of the recombination [9] and from the spin polarization of the triplet state $^3P680^*$ pointing to a radical pair precursor [10]. These kinetic data, linear dichroic measurements [11] and the homology of the amino acid sequence of the D_1 and D_2 proteins of PS II with the L and M subunits of bacterial RCs [12] show PS II and the bacterial RC to be highly analogous.

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Abbreviations: RC, reaction center; PS II, Photosystem II; Chl, chlorophyll a; Pheo, pheophytin a; P680, primary donor in PS II; P, primary donor in bacterial RCs; BChl, bacteriochlorophyll; H, bacteriopheophytin; MARY, magnetic field dependence of the reaction yield; S-T-mixing, singlet-triplet-mixing.

This analogy, however, does not necessarily extend to every structural and functional detail. From an analysis of spectral characteristics and other features it was concluded that P680 is functionally most likely a Chl monomer and not a dimer as the primary donor P in bacterial RCs [13]. Also, the orientations of the ring plane of P680 and of its Q_y-transition moment relative to the membrane seem to be different in PS II compared to bacterial RCs, as concluded from orientation-dependent EPR spectra [14] and from LD-ADMR (Linear-Dichroic Absorbance Detected Magnetic Resonance) measurements [13], respectively. All these results do not exclude that two Chls are in similar positions as the two bacteriochlorophylls (BChl) of P, but are not excitonically coupled. A different orientation of at least one of these two Chls compared to the two BChls of P, as is indicated by the above-mentioned measurements, could indeed be responsible for the lacking excitonic coupling between the two Chls. On the other hand, linear dichroism measurements showed the Q_x-transition moments of both Pheos in PS II to have the same orientation as those of the bacteriopheophytins in bacterial RCs [11].

The recombination of the primary radical pair in bacterial RCs has been used to obtain detailed information about the states involved in primary charge separation, in particular about their energetics and electronic couplings [15–17]. Here we report high resolution magnetic field-dependent measurements of the recombination of $P680^+Pheo^-$ in the temperature range between 90K and 300K. The results allow conclusions on the energetics of primary charge separation and recombination in PS II. In addition, our results point to a close similarity

of the electronic coupling in the primary radical pair in PS II and the bacterial RCs. This confirms the structural similarity of the two RCs and thereby also confirms the functionality of the D_1D_2-preparation of PS II.

2. MATERIALS AND METHODS

$D_1D_2cytb_{559}$ reaction centers were prepared from *Spinacia oleracea* by the methods of Nanba/Satoh [1] and McTavish et al. [2] with slight modifications. The final preparation yielded RCs ($OD_{675\ nm,\ 1\ cm}$ = 18 after ultrafiltration with Amicon Centricons, cut-off 30 kDa) in 50 mM Tris-HCl (pH 7.2), 0.1% (w/v) dodecyl-maltoside, 80 μM Pefabloc (Merck). RCs were stored at 77K. Before the measurement they were thawed on ice, carefully bubbled with cooled N_2 gas for 30 min and diluted with glycerol/buffer (bubbled with N_2 gas for 24 h) to yield a glycerol concentration of 60% (v/v) and an optical density of approx. 1 at 675 nm in a 2 mm cuvette. All steps were carried out on ice under green light in a N_2 atmosphere. Typical absorbance spectra are shown in Fig. 1. The cuvette was sealed with parafilm and transferred into a cryostat containing N_2 gas. In one case the oxygen content was further reduced by four freeze–thaw cycles after which the cuvette was sealed by fusing.

Before and after all time resolved measurements groundstate absorbance spectra were recorded at room temperature. Even extended measurements at 90K did not cause any significant photodamage resulting in a blue shift of the 675 nm absorbance [18]. Measurements at higher temperatures, lasting several hours, caused shifts of this absorbance band by at most 1–2 nm. Time-resolved measurements at 90K before and after each measuring session at higher temperatures showed that these shifts were not accompanied by changes of the kinetics on the nano- to millisecond timescale. However, a decrease of max. 20% of the initial amplitude of the bleaching at 680 nm was observed, mostly due to a decrease of the 100 ns and ms components (see below). This decrease of the amplitude after ageing is comparable to that observed in bacterial RCs under similar conditions (M. Volk et al., unpublished results).

Nanosecond time resolved absorbance measurements were performed as described previously [19]. RCs were excited at 620 nm with a Nd:YAG-laser pumped dye laser (pulse width 2 ns) with an intensity of approx. 0.5 mJ/cm^2 corresponding to a yield of approx. 20% excited RCs. Absorbance changes were probed at various wavelengths with an N_2-laser pumped dye laser (pulse widths 1.5–2 ns). The delay time between excitation and probing pulse was adjusted electronically between 0 ns and 10 ms. A magnetic field of up to 2000 G was applied by a pair of Helmholtz coils with an iron yoke, the magnetic field was calibrated by a gaussmeter with Hall-probe (Bell).

3. RESULTS

Fig. 2 shows the time dependence of the 681 nm absorbance bleaching at 90K. The absorbance partly recovers on the nanosecond timescale, with two time constants of 6–12 ns and 100–110 ns (amplitude ratio approx. $^1/_2$). As discussed below, the faster component can be ascribed to the decay of excited Chls uncoupled from electron transfer and for the fit of the second time constant is fixed to 6 ns, while the slower component mirrors the recombination of $P680^+Pheo^-$. The residual bleaching indicates the formation of $^3P680^*$ (according to the radical pair mechanism, see below), since it decays with a time constant of 1.61 ms, which is characteristic for this state [8]. An external magnetic field of 700G

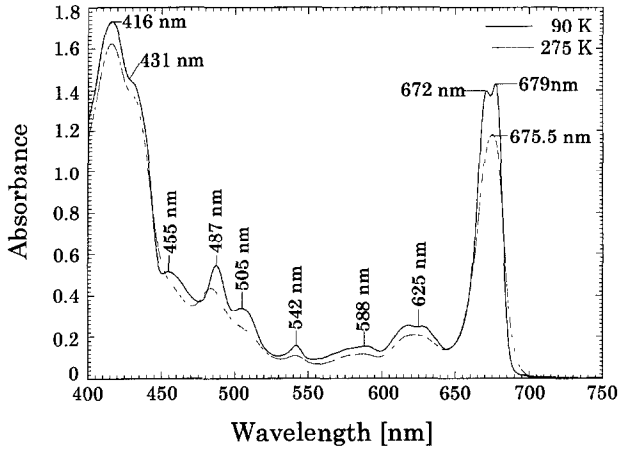

Fig. 1. Absorbance spectra of $D_1D_2cytb_{559}$-RCs of PS II in glycerol/buffer solution at 90K (—) and 290K (.....).

slows the radical pair recombination to 175 ns and decreases the yield of $^3P680^*$, but has no effect on the fast decay of the uncoupled Chls or on the decay time of $^3P680^*$. At 672 nm as well as at the maximum of the Pheo Q_x-absorbance at 544 nm we observed essentially the same time constants for the recombination of $P680^+Pheo^-$ and for the decay of $^3P680^*$. However, no significant 6 ns component is necessary to fit the data at these wavelengths. At all wavelengths a minor component with a time constant of 10–100 μs is observed,

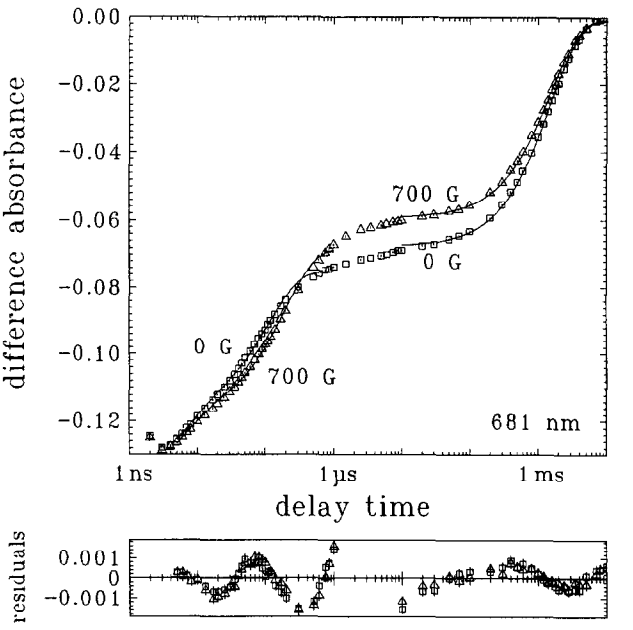

Fig. 2. Time dependent recovery of the absorbance bleaching observed at 681 nm in $D_1D_2cytb_{559}$-RCs of PS II in glycerol/buffer solution at 90K, 0 G (□) and 700 G (△). Solid curves are independent fits of the data at delay times 5 ns–1 μs (biexponential, fast time constant fixed to 6 ns, see text) and 10 μs–10 ms (monoexponential), the results of which are given in Table I. Also shown are the residuals of the fits.

which probably arises from a carotenoid triplet state formed with a yield of 2–3% [8,18,20].

The recovery of the absorbance at 681 nm was measured at different temperatures between 90K and 310K. At all temperatures the fast component with approx. 6 ns lifetime was observed, while the recombination lifetimes as well as the lifetime of ^{3}P680* decrease with increasing temperature. Table I summarizes these results. As described in section 2, even at 310K no significant photodamage affecting the observed kinetics occurred. At 290K we observed an increase of the lifetime of ^{3}P680* from 0.25 ms to 1.02 ms after further reducing the oxygen concentration by four freeze–thaw cycles, in accordance with the values reported in [18,20], while at 250K the lifetime of ^{3}P680* is unaffected by this treatment. At both temperatures, no significant effects of residual oxygen on the recombination of P680$^+$Pheo$^-$ were found, Table I.

The magnetic field dependence of the yield Φ_T of ^{3}P680* (MARY, MAgnetic field dependence of the Reaction Yield) was monitored at 681 nm at a delay time of 5 μs. We found a monotonous decrease of Φ_T with increasing magnetic field, saturating above 1500 G. The halfwidth $H_{1/2}$ of this decrease and the modulation of Φ_T by saturating magnetic fields are given in Table I. In no case we found a resonance in the MARY-curve as was observed for PS I [21] and RCs of *Chloroflexus aurantiacus* [22].

4. DISCUSSION

4.1. *Chlorophylls uncoupled from electron transfer*

The fast component observed at 681 nm with a time constant of 6–12 ns, as obtained from biexponential fits of the data at delay times between 5 ns and 1 μs, is not related to the recombination of P680$^+$Pheo$^-$, but to the decay of excited Chls uncoupled from electron transfer, which has been reported before [7,18,23,24]. This conclusion can be drawn from our results showing that (i) no significant fast component is observed in the Q_x-band of Pheo at 544 nm, (ii) the fast component shows no dependence on the magnetic field and (iii) the ratio of the amplitude of the fast component to that of the 100 ns component increases after ageing of the sample by extended measurements.

4.2. *Radical pair recombination*

Similar to the radical pair P$^+$H$^-$ (P: bacteriochlorophyll dimer, H: bacteriopheophytin) in the bacterial RC [17,25], the radical pair P680$^+$Pheo$^-$ of PS II in the absence of quinone recombines on the 100 ns time scale [7–9]. Recombination proceeds from the originally formed singlet state ^{1}(P680$^+$Pheo$^-$) to the ground state P680 with the rate k_S or after hyperfine induced singlet-triplet-mixing (S-T-mixing) from the triplet phased radical pair to the triplet state ^{3}P680* with the rate k_T [26,27]. At low magnetic fields, S-T-mixing involving all three triplet levels is possible. A magnetic field of several 100 G removes the degeneracy of two of the three triplet radical pair states with the singlet state, thus reducing the yield Φ_T of ^{3}P680* [9].

The radical pair lifetimes and the relative magnetic field dependence of Φ_T given in Table I are in agreement with values reported before [7–9], however, they were determined with much higher precision. In particular, we observed deviations of the recombination of P680$^+$Pheo$^-$ from monoexponentiality, see the residuals of the fits in Fig. 2. These deviations arise from the inherent heterogeneity of the hyperfine induced S-T mixing due to the statistical orientation of the nuclear spins. Similar deviations were observed for the recombination of P$^+$H$^-$ in bacterial RCs [22,28]. Because of these deviations from monoexponentiality, the radical pair recombination cannot be separated unambiguously

Table I

Temperature dependent results of transient absorbance measurements on RCs of PS II at 681 nm (A) on samples containing residual oxygen or (B) after additional freeze–thaw-cycles to remove residual oxygen (see text)

		A						B			
T[K]		90	250	270	280	290	300	250	290	300	310
τ_{RP} [ns]	0G	105	71	60	56	52	n.d.	71	52	49	43
	700G	175	93	77	72	67	n.d.	92	64	59	51
τ_3 [ms]	0G	1.61	1.12	1.05	0.94	0.25	0.33	1.13	1.02	0.97	0.73
	700G	1.61	1.12	1.05	0.95	0.26	0.31	1.12	1.03	0.98	0.74
$H_{1/2}$ [G]		340	230	210	200	190	180	220	200	200	190
$\Phi_T(H)/\Phi_r(0)$		0.84	0.74	0.70	0.68	0.69	0.67	0.74	0.72	0.67	0.70

τ_{RP}, lifetime of P680$^+$Pheo$^-$ (obtained from biexponential fits of the data at delay times 5 ns–1 μs with the faster time constant fixed to 6 ns, see text, uncertainty ± 2 ns, n.d. not determined); τ_3, lifetime of ^{3}P680* (± 0.02 ms); $H_{1/2}$, halfwidth of the magnetic field dependence of the triplet yield (± 10 G); $\Phi_T(H)/\Phi_T(0)$, modulation of the triplet yield by a saturating magnetic field (> 1500 G) (± 0.02).

from the observed fast component by a biexponential fit. Therefore, resting on the assignment of the fast component to uncoupled Chls, its time constant was fixed to 6 ns, the lifetime of the excited state of chlorophyll *a* in solution [29], for determining the (mean) radical pair lifetimes τ_{RP} given in Table I.

The radical pair lifetimes observed in PS II (105 ns at 90K and 52 ns at 290K) are significantly longer than observed in bacterial RCs. In RCs of *Rb. sphaeroides* τ_{RP} decreases from 21 ns to 13 ns upon raising the temperature from 90K to 290K [17]. Almost the same results were obtained for RCS of *Rb. capsulatus* and *Chloroflexus aurantiacus* [22]. The halfwidth $H_{1/2}$ in PS II of 340 G at 90K and 190 G at 290K is larger than that found in bacterial RCs. Between 90K and 290 K $H_{1/2}$ decreases from 55 G to 42 G in RCs of *Rb. sphaeroides* [16] and from 120 G to 55 G in RCs of *chloroflexus aurantiacus* [22].

4.3. *Determination of the recombination rates* k_S *and* k_T

Upon application of an external magnetic field which hinders the triplet recombination channel, the radical pair lifetime increases. This shows that in PS II, as in bacterial RCs [17], the singlet recombination rate k_S is smaller than the triplet recombination rate k_T.

The width of the magnetic field dependence of Φ_T (MARY-halfwidth) $H_{1/2}$ is determined by the hyperfine interaction, the radical pair singlet-triplet splitting J and the lifetime broadening of the radical pair states [26,30,31]. The hyperfine interaction, as estimated from the width of the EPR-line of 8 G for $P680^+$ [32] and 13 G for $Pheo^-$ [33], is too small to account for the large values of $H_{1/2}$ of 180–340 G. Similarly, the singlet-triplet-splitting can be estimated to be smaller than approx. 50 G from the lack of a resonance structure in the MARY-curve. Therefore, the main contribution to $H_{1/2}$ has to arise from a lifetime broadening corresponding to a depopulation rate of several ns^{-1}. The backward electron transfer $P680^+Pheo^- \rightarrow {}^1P680^*$ is activated and can be ruled out as the major contribution to the lifetime broadening because of the increase of $H_{1/2}$ upon lowering the temperature. It can be concluded, that the sum of the recombination rates k_S and k_T is larger than 1 ns^{-1}. Since the radical pair lifetime is on the order of 100 ns, on the other hand, one of the rates must be much smaller than 1 ns^{-1}. From the magnetic field dependence of τ_{RP}, k_S was concluded to be smaller than k_T. Therefore, the dominant contribution to $H_{1/2}$ arises from k_T, showing k_T to have a value of several ns^{-1}, with the S-T-mixing constituting the effective bottleneck for fast triplet recombination. The determination of the exact value of k_T from the magnetic field dependent recombination measurements involves the solution of the stochastic Liouville equation with the relevant spin Hamiltonian [26,31].

The value of k_T estimated from $H_{1/2}$ increases from 3 ns^{-1} at 290K to 6 ns^{-1} at 90K. Preliminary results of the thorough treatment yield only slightly smaller values of k_T. The triplet recombination rate k_T shows an inverted temperature dependence, increasing by a factor of 2 upon lowering the temperature from 290K to 90K. This is the same dependence as found for k_T in the RC of *Chloroflexus aurantiacus* [22] and very similar to that found for the RCs of *Rb. sphaeroides* [16] and *Rb. capsulatus* [22]. The inverted temperature dependence of k_T shows that k_T is activationless, implying similar values for the free energy gap ΔG_T and the reorganization energy λ_T for $P680^+Pheo^- \rightarrow {}^3P680^*$ [34].

The singlet recombination rate k_S in principle can be determined from the magnetic field dependence of τ_{RP} and Φ_T [17,22]. Values of 0.002 ns^{-1} at 90K and 0.01 ns^{-1} at 290K are obtained, smaller than the values found in bacterial RCs [17,22]. However, the deviations of the radical pair recombination from monoexponentiality due to the statistical orientation of the nuclear spins impede this direct determination of k_S in PS II. The value of τ_{RP} needed for the determination of k_S is the average over all nuclear spin orientations. In PS II the difference between k_S and k_T is much larger than in bacterial RCs, therefore the distribution of radical pair lifetimes, ranging approx. $2/(k_S + k_T)$ (fast S-T-mixing) to $1/k_S$ (no S-T-mixing) [22], is much broader. Numerical simulations show that in this case almost the same distribution of radical pair lifetimes is expected when varying k_S between 0.002 ns^{-1} and 0.0002 ns^{-1}, with the position of the maximum of the distribution shifting only slightly. Monoexponential fits of the time dependence of the recombination calculated with these lifetime distributions yield almost the same time constants, although the average lifetimes differ by almost one order of magnitude. Therefore, the values of τ_{RP} in Table I, obtained from monoexponential fits of the data, not necessarily reflect the true average lifetimes and do not allow an exact determination of k_S. A better fitting procedure taking into account the distribution of lifetimes is in preparation. Preliminary results from simulations, employing the stochastic Liouville equation, of the magnetic field modulation of the triplet yield, which also is sensitive to k_S, indeed indicate that k_S has values around 0.0004 ns^{-1} at 90K and 0.0015 ns^{-1} at 290K.

These values for k_S are smaller than those found in bacterial RCs [17,22] by a factor 50. In contrast to the bacterial RC, it cannot be excluded, that in PS II a significant amount of the singlet recombination proceeds via thermal repopulation of $^1P680^*$ and deactivation of the excited state. Therefore, the rate of k_S of direct recombination from $P680^+Pheo^-$ to the ground-state might be even smaller than the values estimated here for the overall recombination from the singlet radical pair.

4.4. *Energetics of* $P680^+Pheo^-$ *at 290K (Fig. 3)*

In bacterial RCs at higher temperatures, $^3P^*$ partly decays via thermal repopulation of P^+H^- with a rate

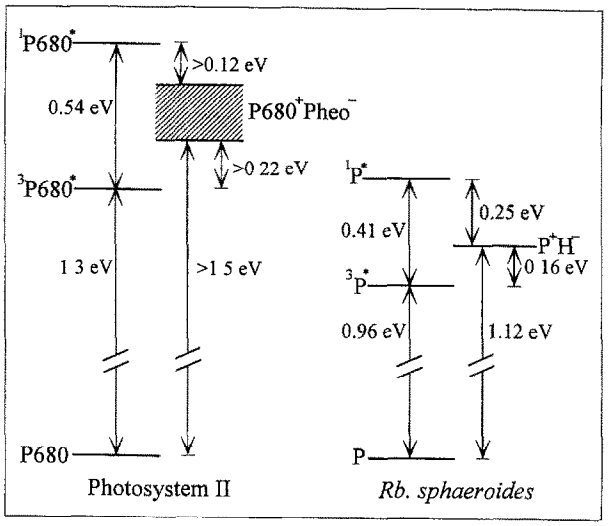

Fig. 3. Comparison of the energies of ^{1}P680* and ^{3}P680* and P680$^+$Pheo$^-$ at 290K in Photosystem II, estimated as described in the text, with those of ^{1}P*, ^{3}P* and P$^+$H$^-$ in RCs of *Rb. sphaeroides* [17].

k_{-T}, followed by triplet-singlet-mixing and recombination via k_S. The magnetic field dependence of this ^{3}P* decay channel allows its separation from direct intersystem crossing and the determination of ΔG(P$^+$H$^-$-^{3}P*) from k_{-T}/k_T [15,17]. Since in PS II we observed no magnetic field dependence of the lifetime of ^{3}P680*, only a lower limit of ΔG(^{3}P680*-P680$^+$Pheo$^-$) of 0.22 eV at 290K can be estimated from our data and absolute triplet yield of approx. 0.3 [8,18,20]. With the energy of ^{3}P680* of 1.3 eV, as estimated from the wavelength of phosphorescence [35], this yields a lower limit of ΔG(P680$^+$Pheo$^-$-P680) of 1.5 eV.

An estimate of ΔG(^{1}P680*-P680$^+$Pheo$^-$) can be obtained from the following argument: the free energy of P680$^+$Pheo$^-$ necessarily is below that of ^{1}P680*, as can be concluded from the fast charge separation at high and low temperatures [4,5,36] which only is possible for an electron transfer rate near the activationless case, implying a negative free energy gap ΔG(^{1}P*-P$^+$H$^-$). Therefore, the backward electron transfer P680$^+$Pheo$^-$ $\rightarrow$ ^{1}P680* with the rate k_{-1} is activated and decreases upon lowering the temperature. k_{-1} may contribute to the lifetime broadening of the singlet radical pair and, therefore, to the MARY-halfwidth $H_{1/2}$. However, k_{-1} cannot be the major contribution to $H_{1/2}$ which increases upon lowering the temperature. Allowing for a maximum contribution of 60 G to $H_{1/2} \simeq 200$ G from k_{-1}, k_{-1} is restricted to values smaller than 1 ns^{-1}. Comparing this with the reported value $k_1 = (10\ \text{ps})^{-1}$ for the rate of primary charge separation [36], a lower limit of $|\Delta G(^1\text{P680*-P680}^+\text{Pheo}^-)| > 0.12$ eV at 290K can be estimated**. With an energy of ^{1}P680* of 1.84 eV, as estimated from the fluorescence spectrum [23], this yields an upper limit of ΔG(P680$^+$Pheo$^-$-P680) of 1.72 eV.

4.5. *Comparison of the recombination rates in Photosystem II and bacterial reaction centers*

Since the triplet recombination rate k_T is activationless in PS II and in the RCs of *Rb. sphaeroides*, *Rb. capsulatus* and *Chloroflexus aurantiacus*, the value of k_T allows to conclude upon the value of the electronic coupling V_T. This procedure is justified, since the energetics, as discussed above, are similar and the activationless rates imply $\tau_T \simeq -\Delta G_T$. The value of k_T of 3 ns^{-1}, together with $k_T \simeq 0.6$–0.8 ns^{-1} and $V_T \simeq 1$ cm^{-1} in the bacterial RCs [16,22,37], yields a value of V_T of approx. 2 cm^{-1} in PS II.

The singlet recombination rate k_S in PS II is significantly smaller than in bacterial RCs by a factor of at least 50. The electronic coupling for the singlet recombination process should be similar to that for the triplet recombination and, therefore, is expected to be larger in PS II than in the bacterial RCs. Thus, the difference of the rates must be due to the Franck–Condon factor. With k_S being a rate in the Marcus inverted region, this difference originates from the larger free energy of P680$^+$Pheo$^-$ of more than 1.5 eV compared to that of P$^+$H$^-$ of 1.12 eV [17], the difference being mostly due to the different redox potentials of the primary donors (P680/P680$^+$: $\geq$ +800 mV [38]; P/P$^+$: +440 mV [39]).

5. CONCLUSIONS

Due to the different chemical nature of the primary donors employed in PS II and bacterial RCs, the free energy of the primary radical pair in PS II is larger than that in bacterial RCs. The immediate consequence of this difference is that the recombination rate to the groundstate in PS II is deeper in the Marcus inverted region than in the bacterial RCs. This is reflected in the slower rate k_S and the resulting long lifetime of the radical pair in PS II.

Our analysis shows the electronic coupling V_T for the recombination of the primary radical pair to be similar in PS II and bacterial RCs. The values of V_T essentially are determined by the distances and the relative orientations of P680 and Pheo and of P and H. Since the electronic coupling depends exponentially on the distance [34], the observed similarity of V_T in PS II and bacterial RCs suggests that the relevant distances between the cofactors are also similar. Structural differences in the primary donor as reported in [13,14] could only have a minor influence on V_T. Therefore, we conclude that our results support the notion that the structure of PS II is similar to that of the bacterial RC.

On the other hand, this similarity of the electronic

** In [24] a value of 0.124 eV was calculated for the absolute value of ΔG(^{1}P680*-P680$^+$Pheo$^-$) from the fastest and the slowest fluorescence component at 277K. However, due to the limited time resolution and difficulties in the assignment of the fast fluorescence components [6], this agreement might be accidental.

couplings in the D_1D_2-preparation of PS II and the bacterial RCs investigated so far also seems to confirm the functionality of the D_1D_2-preparation. In this context, it is also interesting to note, that the RC of *Chloroflexus aurantiacus*, which functionally closely resembles the RC of *Rb. sphaeroides*, is as minimized as the D_1D_2-RC of PS II with respect to the protein subunits [40].

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