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High quantum yield of charge separation in reaction centers of *Chloroflexus aurantiacus*

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The relative quantum yield of charge separation in photosynthetic reaction centers of *Chloroflexus aurantiacus* and *Rhodobacter sphaeroides* was measured on the nanosecond timescale. Thereby the quantum yield becomes independent of a potential loss of both quinones during reaction center isolation. Based on the quantum yield of 1.02 ± 0.04 for reaction centers of *Rhodobacter sphaeroides* at room temperature (Wraight, C.A. and Clayton, R.K. (1973) Biochim. Biophys. Acta 333, 246–260), the quantum yield of initial charge separation in reaction centers of *Chloroflexus aurantiacus* at 280 K was determined to be 1.06 ± 0.11.

Introduction

One of the impressive features of the primary charge separation in photosynthetic reaction centers (RCs) of purple bacteria is the high quantum yield (QY) for the photoinduced transmembrane transfer of an electron. This occurs in a sequence of electron transfer (ET) reactions along one of the two pigment branches of the RC, the A-branch, as revealed by transient spectra [1,2] in combination with X-ray structural data [3–5] and polarized spectroscopy [6]. Photoinduced ET takes off from the initially excited bacteriochlorophyll dimer ($^1P^*$) and leads to a reduced bacteriopheophytin (H) and subsequently to the reduction of the primary quinone (Q). For *Rb. sphaeroides* R-26 the QY of charge separation to P^+Q^- was measured to be 1.02 ± 0.04 for excitation in the Q_y-transition of P at room temperature [7].

In contrast, a lower QY of 0.61 ± 0.05 has been reported for the green bacterium *Chloroflexus aurantiacus* under the same conditions of excitation and temperature [8]. Although a crystal structure analysis is not yet available for this RC, the combination of other features as cofactor content [9,10], primary sequence of the two protein subunits [11–13] and spectroscopic evidence [14] including Stark spectroscopy (Eberl, U., Braun, H.P. and Michel-Beyerle, M.E., unpublished data) allows the conclusion that also this RC has two pigment branches, A and B. As in purple bacteria, the cofactors at the A-branch follow the sequence P-B-H-Q_A (with B denoting the bacteriochlorophyll (BChl) monomer). At the position of B at the B-branch a bacteriopheophytin (BPh) is bound, however. As in the case of *Rb. sphaeroides*, one of the two quinones is lost during the isolation of RCs. This is expected to be Q_B for two reasons: (i) the homology of the primary sequence of the Q binding sites in *Chloroflexus* [11–13] and *Rb. sphaeroides* [15] and (ii) the fact, that the approx. 60 ms recovery of the bleaching of P is slowed to approx. 550 ms after addition of naphthoquinone, together with the fact that the fast recovery is restored after further addition of *o*-phenanthroline, which blocks ET between Q_A and Q_B [10].

The low QY of P^+Q^- formation in *Chloroflexus* could arise from one of the following effects: (1) deactivation of $^1P^*$ along routes competing with charge separation or recombination of intermediate radical pairs at the B- (2) or the A-branch (3) or (4) the loss of Q_A.

Abbreviations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; P, special pair; Q, quinone; RC, reaction center; LDAO, lauryldimethylamine oxide; PVA, poly(vinyl alcohol); QY, quantum yield; ET, electron transfer.

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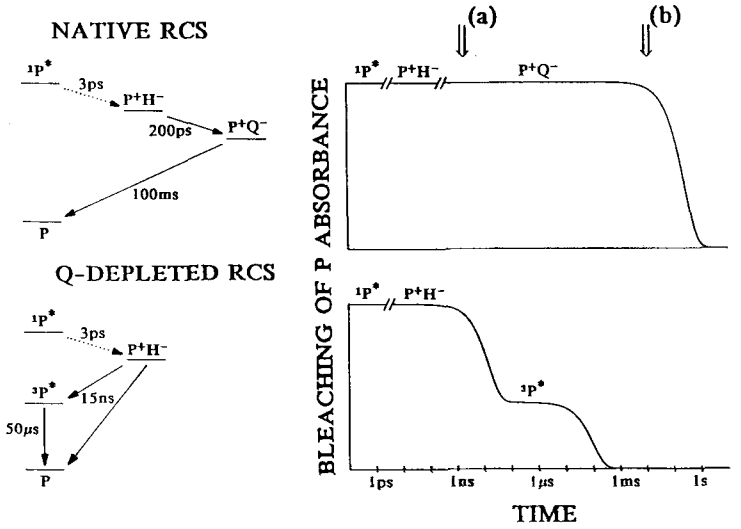

Fig. 1. (Left) Reaction scheme for charge separation and recombination in native (Q-containing) and Q-depleted RCs. (Right) Scheme of QY measurements: (a) this work, (b) [7,8].

(1) Appreciable losses from $^1P^*$, e.g., via internal conversion, can be excluded in RCs of *Chloroflexus*, since in femtosecond experiments the longest component of the stimulated emission from $^1P^*$ has been shown to be 7 ps at room temperature, whilst the bleaching of the P ground-state absorbance does not change during this time [16,17].

(2) The possibility of ET to the B-branch followed by recombination is one of the interesting questions with respect to the *Chloroflexus* RC. The proximity of BPh to P could in principle promote charge separation also along the B-branch. Indeed, a negative difference absorbance is observed at 533 nm, in the spectral range of the Q_x transition of the two BPhs at the B-branch with a lifetime of approx. 300 ps [18,19]. If this were indeed to indicate ET to the B-branch, recombination of the radical pair to the ground state prior to the millisecond measurements employed in Ref. 8 could explain the low quantum yield of 0.6 observed there. Alternatively, the effect has also been discussed as an electrochromic shift or a heterogeneity with respect to the absorbance of the active BPh.

(3) Recombination of P^+H^- has to be considered only if in RCs with functional Q_A recombination were to be faster than approx. 2 ns, so that it could effectively compete with the forward ET to Q_A, which proceeds in 200–300 ps [18,20].

(4) The apparent low QY reported for *Chloroflexus* could also reflect the loss of Q_A, since it was derived from the induction rate of photooxidation of P at the beginning of steady state illumination on the millisecond time-scale. The fraction of Q-free RCs leads to an underestimation of the QY, since in the absence of Q no P^+Q^- can be formed. Instead, in Q-free RCs the precursor state P^+H^- recombines on the 10 nanosecond time-scale [21,22], partly to the groundstate PH, partly to the triplet state $^3P^*$ having a lifetime of approx. 50–80 μs [23,24] (Fig. 1). None of these intermediate states can be populated appreciably under steady-state conditions. In fact, the lower QY reported for *Chloroflexus* was suspected to be due to "insufficient electron transfer to quinones, which were partially removed from the reaction centers during the purification and replaced by ubiquinone$_6$" [8]. We have observed that, in general, in all preparations a certain amount of RCs have lost both quinones (Scheidel, G., Häberle, T., Volk, M., Ogrodnik, A., Feick, R. and Michel-Beyerle, M.E., unpublished data).

It is the goal of this paper to reexamine the QY of charge separation in RCs of *Chloroflexus aurantiacus* and to detour potential complications due to loss of Q_A by measurements on the nanosecond time-scale. In contrast to measurement on the millisecond timescale, this approach allows us to distinguish between a reduction of the QY due to processes competing with P^+Q^- formation, such as processes (1)–(3), all occurring faster than the time resolution of the experimental apparatus, and preparational artefacts (4), leading to recombination losses on the 10 ns timescale (Fig. 1).

Materials and Apparatus

Q_A-containing RCs of *Rb. sphaeroides* R-26 were prepared from the membrane as described previously [21], except that 0.1% Triton X-100 was employed as detergent instead of lauryldimethylamine oxide (LDAO). The preparation of RCs of *Chloroflexus aurantiacus* in 0.2% LDAO was performed as in Ref. 25, with the exception that the last purification step was omitted.

In order to avoid the artefact of a prolonged bleaching of the P groundstate absorbance [22], RCs were

embedded in poly(vinyl alcohol) films (PVA, Wacker, M_r 40000) of approx. 150 μm thickness at a concentration of typically 100 μM. For *Rb. sphaeroides* two layers of PVA film were used, yielding an absorbance of 0.45 at 865 nm, for *Chloroflexus* three layers gave an absorbance of 0.40 at 865 nm. To improve the optical quality of the samples, the films were mounted between glass plates and glycerol was applied between the layers for refractive index matching. The samples were placed side by side in the same holder, so that they were easily changed by moving the holder orthogonal to the laser beams.

Difference absorbance measurements were carried out with two dye lasers (pulse width 1.4 ns) being pumped by an N_2-laser. The RCs were excited at 600 nm with an energy density of approx. 0.5 mJ/cm^2, which was adjusted to yield about 30% excited RCs/pulse. The diameter of the excitation laser beam on the sample was about 1 mm. The rate of excitation was 2 Hz, ensuring complete P^+Q^- recombination before each excitation pulse.

The bleaching of P absorbance was detected 4 ns and 92 ns after excitation with the other dye laser at 875 nm using a variable optical delay. This laser beam had a diameter of 0.2 mm on the sample and its overlap with the maximum of the excitation profile was individually adjusted for each sample. Values from 100 measurements were averaged after correcting each single measurement for the fluctuating energy of excitation. Each measuring cycle was repeated at least 20 times.

TABLE I

Experimental results

(A) Absorbance measurements: A_{exc}, A_P and A_{865} denoting the absorbance at 600 nm (wavelength of excitation), at 875 nm (probing wavelength) and at 865 nm.

(B) Difference absorbance measurements and quantum yield.

N energy of excitation (a.u.) **. $\phi^*_{av,Sph}/\phi^*_{av,Chl}$ ratio of excitation probabilities, calculated from Eqn. 5. $\Delta A(t)$ bleaching of P ground-state absorbance at 875 nm, at a delay time t between the excitation and the probing pulse. $\Delta A_{P^+Q^-}$, $\Delta A_{P^+H^-}$ bleaching of P ground-state absorbance at 875 nm due to RCs with and without Q, respectively, immediately after excitation, Eqn. 1. $\Delta A_P(0\text{ ns})$ total bleaching of P ground-state absorbance immediately after excitation ($= \Delta A_{P^+Q^-} + \Delta A_{P^+H^-}$). $\Delta A_{Chl}/\Delta A_{Sph}$ ratio of $\Delta A_P(0\text{ ns})$, corrected for the different excitation probabilities ϕ^*_{av}. QY_{Chl}/QY_{Sph} ratio of quantum yields, determined from Eqn. 3.

(A) Absorbance measurements

	Rb. sph.	*Chlorofl.*
A_{exc}	0.276	0.179
A_P	0.415	0.368
A_{865}	0.450	0.395

(B) Difference absorbance measurements and quantum yield

Polarization of exciting and probing pulses	Parallel		Orthogonal	
	Rb. sph.	*Chlorofl.*	*Rb. sph.*	*Chlorofl.*
N (a.u.)	585	656	672	752
$\phi^*_{av,Sph}/\phi^*_{av,Chl}$	1.05		1.055	
ΔA(4 ns)	−0.0730	−0.0695	−0.0647	−0.0611
ΔA(92 ns)	−0.0662	−0.0665	−0.0590	−0.0596
$\Delta A_{P^+Q^-}$	−0.0628	−0.0648	−0.0563	−0.0587
$\Delta A_{P^+H^-}$	−0.0124	−0.0056	−0.0101	−0.0029
$\Delta A_P(0\text{ ns})$	−0.0752	−0.0704	−0.0664	−0.0616
$\Delta A_{Chl}/\Delta A_{Sph}$	0.983		0.979	
QY_{Chl}/QY_{Sph}	1.108		1.104	

Methods and Results

Determination of the relative quantum yield

The quantum yield of P^+-formation reported in this paper is deduced from the amount of bleaching of the P ground-state absorbance after a nanosecond laser pulse. Comparing the relative bleaching of P in RCs of *Chloroflexus* with that in *Rb. sphaeroides* permits determination of the quantum yield of charge separation in *Chloroflexus*.

In the nanosecond range the difference absorbance at 875 nm due to the bleaching of P shows the following time dependence:

$$\Delta A_P(t) = \Delta A_{P^+Q^-} + \Delta A_{P^+H^-}[\phi_T + (1-\phi_T)\,e^{-t/\tau_{Rp}}] \quad (1)$$

where

$\Delta A_{P^+Q^-}$ = bleaching due to Q containing RCs: P^+Q^- is formed in approx. 200–300 ps [19,20,26] and decays in approx. 100 ms [10,27,28];

ΔA_{P+H^-} = initial bleaching due to RCs having lost Q: P^+H^- is formed in 3–20 ps [16,17,29,30] and recombines with τ_{Rp} to PH or $^3P^*H$ (lifetime of $^3P^*$ approx. 50–80 μs [23,24]);

τ_{Rp} = lifetime of radical pair P^+H^- in Q-depleted RCs;

ϕ_T = yield of $^3P^*$ formed by recombination of P^+H^- in Q-depleted RCs. We have measured the triplet yield, ϕ_T, and the radical-pair lifetime τ_{Rp} on Q-depleted RCs to be 0.31 and 15 ns for *Chloroflexus* and 0.27 and 15 ns for *Rb. sphaeroides* at 290 K in PVA films (our unpublished data), the latter values being very similar to those measured in solution [21,31].

From the measured bleaching at 4 ns and 92 ns after excitation together with the radical-pair lifetime it is possible to extrapolate to the initial amplitude of the bleaching $\Delta A_P(0\text{ ns})$. With the known triplet yields the value of $\Delta A_{P^+Q^-}$ and $\Delta A_{P^+H^-}$ can be determined separately, Table I(B). The ratio of these mirrors the ratio of Q-containing to Q-free RCs; in our samples there were about 16% Q-free RCs in the case of *Rb. sphaeroides* and about 6% in the case of *Chloroflexus*.

** Values for parallel and orthogonal polarization of exciting and probing pulses are not comparable.

The quantum yield of P^+-formation on the nanosecond timescale is given by the bleaching of the P ground-state absorbance at $t = 0$ ns, ΔA_p (0 ns), normalized to the P groundstate absorbance, A_P, at the probing wavelength and corrected for the (non-saturating) excitation probability, ϕ^*_{av}.

$$QY = \frac{\Delta A_P(0\text{ ns})/A_P}{\Delta\epsilon_{pr}/\epsilon_{pr}\phi^*_{av}} \tag{2}$$

Here ϵ_{pr} denotes the extinction coefficient and $\Delta\epsilon_{pr}$ the difference extinction coefficient, accounting for the fact that the bleaching of P absorbance at 865 nm is not complete [32]. Due to absorption of the Bph anion, the values of $\Delta\epsilon_{pr}$ for the states P^+Q^- and P^+H^- actually are different. The exact value of the extinction coefficient of H^- in the RC at 865 nm is not known; from the difference spectrum of BPh^-/BPh in solution [33], however, it can be estimated that it amounts to at most 15% of the extinction coefficient of P at 865 nm. Because of this small contribution of H^- to $\Delta\epsilon_{pr}$ and the small content of Q-free RCs in our samples, the error in the final result of QY introduced by assuming the same value of $\Delta\epsilon_{pr}$ for P^+Q^- and P^+H^- in Eqn. 1 amounts to less than 1%.

As shown below, the absolute value of ϕ^*_{av} could not be determined exactly, only the ratio for the measurements on *Rb. sphaeroides* and *Chloroflexus*. Therefore we calculated the ratio of quantum yields for P^+ formation:

$$\frac{QY_{Chl}}{QY_{Sph}} = \frac{(\Delta A_p(0\text{ ns})/A_p)_{Chl}\phi^*_{av,Sph}}{(\Delta A_p(0\text{ ns})/A_P)_{Sph}\phi^*_{av,Chl}} \tag{3}$$

Here, $\Delta\epsilon_{pr}/\epsilon_{pr}$ was assumed to be the same in the two samples (Appendix II).

Excitation probability

For comparing the measured bleaching of P ground-state absorbance in RCs of *Rb. sphaeroides* with that of *Chloroflexus* the different probability of excitation of a RC after an actinic light pulse has to be known for the two samples. The excitation probability, ϕ^*, is given by the intensity, N (number of photons per area), of the actinic pulse and the extinction coefficient ϵ_{exc} of the sample at the excitation wavelength (here 600 nm) [×].

$$\phi^* = 1 - e^{-\sigma N} \tag{4}$$

Here, σ is the cross-section for the absorption of a photon, which can be calculated from ϵ_{exc} according to $\sigma = \epsilon_{exc}\ \ln(10)/L$, L denoting Avogadro's constant.

As the actinic light is being absorbed in the sample, its intensity and thereby the probability of excitation decrease in the sample, higher concentrations leading to a faster decrease. With the absorbance A_{exc} at the excitation wavelength this effect can be estimated, giving the averaged excitation probability ϕ^*_{av} (Appendix I):

$$\phi^*_{av} = 1 - \frac{1}{A_{exc}\ln 10}\int_{10^{-A_{exc}}}^{1}\frac{e^{-\sigma N y}}{y}\,dy \tag{5}$$

For measuring the absorbance of the samples, they were mounted in the holder beside the equivalent number of layers of PVA-film without RCs as a reference. The absorbance was measured at 600 nm, 865 nm and 875 nm with the excitation laser and the probing laser, respectively, both at low intensities, detecting pulse energies before and behind the sample. Calibration of the detectors was done with the reference PVA films. The results from 100 laser flashes were averaged, each measurement including calibration was repeated five times. The results are given in Table I(A).

The extinction coefficients of the PVA samples at 600 nm could be evaluated from the measured absorbances at 600 nm and 865 nm and the extinction coefficients at 865 nm in aqueous solution. With $\epsilon_{865} = 128\ mM^{-1}\ cm^{-1}$ [32] we determined $\epsilon_{600} = 78\ mM^{-1}\ cm^{-1}$ for RCs of *Rb. sphaeroides* and with $\epsilon_{864} = 130\ mM^{-1}\ cm^{-1}$ [34] $\epsilon_{600} = 59\ mM^{-1}\ cm^{-1}$ for RCs of *Chloroflexus*. The equivalency of the extinction coefficients at 865 nm in PVA and aqueous solution is supported by identical absorption spectra. Distortions of the spectra by intensive drying of the PVA films were avoided. In any way, only the ratios of the extinction coefficients at 600 nm for *Rb. sphaeroides* and *Chloroflexus* will be used later, so that possible differences of the extinction coefficients in PVA and aqueous solution would cancel, as long as they were similiar for both species.

Though detecting the energies of the excitation laser pulses with a precision of approx. 1%, the absolute values of the intensity in the maximum of the spatial excitation profile could not be determined accurately, since the size and intensity distribution of the laser beam could not be measured with the same accuracy. However, by mounting the two samples in one holder and by always adjusting the overlap of the probing and the exciting laser beams, we made sure that the intensity distribution was the same in both samples, so that the ratio of intensities is given by the ratio of the measured pulse energies.

Taking the measured extinction coefficients at 600 nm and the measured ratio of excitation energies the

[×] Eqns. 4 and 5 do not take into account polarization effects. These, however, cancel in calculating the ratio of excitation probabilities for the two samples, since this ratio does not depend on the absolute values of σN, as discussed later.

TABLE II

Uncertainties of measurement

Standard deviation of difference absorbance from up to 20 measurements	1%
Standard deviation of absorbance from five measurements was 1%, effects of scattering in PVA films suggest a higher value of uncertainty for A_{pr} of at most	5%
Uncertainty of determination of the relative excitation energies was better than	1%
Errors in the determination of the extinction coefficient at 600 nm (deduced from the absorbances at 865 nm and 600 nm) and of the absorbance at 600 nm partly cancel in Eqn. 5, thus suggesting an overall uncertainty of ϕ^*_{av} of at most	5%
Uncertainty of the equality of $\Delta\epsilon_{pr}/\epsilon_{pr}$ (estimated from the difference in ϵ_{865} [34], Appendix II)	2%
Uncertainty of the radical-pair lifetimes τ_{Rp} (± 2 ns) yields an error smaller than (errors in the the triplet yield ϕ_T have no effect on the value of ΔA_P (0 ns))	1%
Square root of sum of the squares of the above: (uncertainty for measurement in one sample)	7.5%
Uncertainty of the ratio of measurements in two samples:	$7.5\% \cdot \sqrt{2} = 10.6\%$

ratio $(\sigma N)_{Sph}/(\sigma N)_{Chl}$ is given, but not the absolute value of (σN) for each sample. It can be shown, however, that, taking values for $(\sigma N)_{Sph}$ and $(\sigma N)_{Chl}$ in the range 0.1–0.5 (corresponding to about 10–41% excited RCs/pulse), the ratio of averaged excitation probabilities $\phi^*_{av,Sph}/\phi^*_{av,Chl}$, calculated from Eq. 5, depends only on the ratio and not on the absolute values of $(\sigma N)_{Sph}$ and $(\sigma N)_{Chl}$ in our case.

Since it is desirable to measure both samples with the same excitation probability, the excitation pulse energy was adjusted for each sample to correct approximately for the difference in extinction coefficients and absorbance, Table I(B). Because of fluctuations and drifts of the laser pulse energy (about 5%), it was not possible to obtain exactly the same excitation probability. Single measurements were therefore normalized to the energy of excitation as described above.

Anisotropy of difference absorption

Because it was not possible to excite and probe the RCs at the same wavelength, linear dichroism has to be considered. Preferentially RCs with the Q_x transition moments of P or the BChl monomers in the direction of the excitation polarization are excited at 600 nm, whilst the measured bleaching of P is mostly due to RCs with the Q_y transition moments in the direction of the probing polarization. Therefore the measured bleaching depends on the relative orientation of the polarization of actinic and probing light as well as on the angle between the transition moments. The 600 nm absorbance band reflects the Q_x transitions of the two BChl monomers and P in RCs of *Rb. sphaeroides*, while there is only one BChl monomer in addition to P in RCs of *Chloroflexus*. This could cause different relative orientations of the effective transition moments for actinic and probing light in the two samples, even if the orientation of the cofactors was the same.

We measured the difference absorbance with parallel and orthogonal polarization of the exciting and probing pulses, using a half-wave plate and a polarizer in the path of the exciting pulse, Table I(B). This also changed the ratio of reflection to transmission of the beamsplitter for the excitation pulses, so that the measurements of the excitation energies, and thereby also of the absolute values of the difference absorbance, with parallel and orthogonal polarization, cannot be compared directly. Thus, no direct information on the anisotropy of the P bleaching could be obtained. However, the ratio of the difference absorbances in the two samples, corrected for the excitation probabilities ϕ^*_{av}, is the same for parallel and orthogonal polarization of probing and exciting light, Table I(B). From that it can be concluded that the anisotropy has to be the same in RCs of *Rb. sphaeroides* and *Chloroflexus*, allowing the use of Eqn. 3 for both polarizations separately. Recent measurements on RCs of *Chloroflexus* and *Rb. sphaeroides* in solution at 290 K, using a refined apparatus, have indeed shown the anisotropy for excitation at 600 nm and probing in the dimer band to be -0.1 in both RCs (Volk, M., Scheidel, G., Häberle, T., Ogrodnik, A. and Michel-Beyerle, M.E., unpublished data).

Results

Inserting the experimental values of Table I(B) for the difference absorbances and absorbances at 875 nm and the ratio of excitation probabilities the following result for the QY of P^+ formation is obtained:

$$\frac{QY_{Chl}}{QY_{Sph}} = 1.11 \pm 0.11$$

Because of the anisotropy of the difference absorbance being the same in *Rb. sphaeroides* and *Chloroflexus*, the measurements with parallel or orthogonal polariza-

tion of exciting and probing light yield the same result. An estimation of the uncertainties of the measurement is given in Table II.

Discussion

The ratio of the quantum yield of charge separation on the nanosecond timescale in RCs of *Chloroflexus* and *Rb. sphaeroides* for excitation at 600 nm has been determined here to be 1.11 ± 0.11. The QY for *Rb. sphaeroides* was measured to be 1.02 ± 0.04 for excitation at 880 nm, reducing by a factor of 0.96 for excitation at 600 nm [7] [×]. Taking the QY for *Rb. sphaeroides* for excitation at 880 nm to be 1, the largest value possible, our measurements yield a value for the QY in *Chloroflexus* for excitation at 600 nm of 1.06 ± 0.11.

This result rests on the assumption that the OY of near unity in *Rb. sphaeroides*, which was obtained for RCs in buffer solution, also holds for RCs in PVA. All ET rates measured both in buffer solution and PVA are very similiar in the two media, differing by at most a factor of 2. These are the rate of primary charge separation from $^1P^*$ [36], the rate of ET from H^- to Q [18,26], the recombination rate from P^+Q^- to the ground-state [27,28] as well as the recombination dynamics of P^+H^- in Q-free RCs (our unpublished data). Similiar differences in the rates of possible loss channels in PVA and solution could not considerably affect a QY of about 1. It also has been shown [37] that, in RCs of *Rb. sphaeroides* which had been allowed to dry on quartz plates to form films, the QY changes only after extensive dehydration, accompanied by considerably changes in the ground-state absorbance spectrum, similar to those observed in extensively dried PVA films. As already mentioned, the PVA films employed here had not been dried extensively and did not show any distortions of the groundstate spectra, compared to RCs in solution. Also for *Chloroflexus* the rate of ET to the Q [18,20], the recombination from P^+Q^- (Aumeier, W., personal communication) and the recombination from P^+H^- in Q-free RCs (our unpublished data) all differ by at most a factor of two in PVA and aqueous buffer solution. We therefore conclude that the result for the QY in *Chloroflexus* obtained here in PVA also holds for RCs in buffer solution. A direct determination of the QY in buffer solution is hindered by the observed prolonged bleaching of the P groundstate absorbance after illumination, which in the case of *Chloroflexus* recovers only after hours, whereas the recovery in *Rb. sphaeroides* is much faster.

The low QY of approx. 0.6 measured on the millisecond timescale [8] has to be attributed to this bleaching effect or to RCs depleted of Q_A. Independent biochemical controls have indeed shown that, under certain conditions, both menaquinones in *Chloroflexus* are easily lost during preparation, probably due to the absence of the H-protein subunit. For example, when Triton X-100 is used as detergent, up to 80% of RCs lose both quinones. However, no increased loss of the primary quinone is observed when preparing RCs of *Chloroflexus* with LDAO as detergent according to the procedure used here. Preparations of RCs of *Rb. sphaeroides* with Triton X-100 or LDAO as detergent typically show a portion of 10–20% RCs depleted of both quinones (Scheidel, G., Häberle, T., Volk, M., Ogrodnik, A., Feick, R. and Michel-Beyerle, M.E., unpublished data).

Conclusions

The determination of the quantum yield of P^+ formation on the nanosecond time scale is insensitive to the occupancy of the quinone binding sites, especially of Q_A. This method has been applied to RCs of *Chloroflexus aurantiacus* for which measurements on the millisecond time scale yielded a quantum yield of about 0.6 [8]. The nanosecond time-resolved investigations reported in this paper, however, indicate that also in *Chloroflexus* the quantum yield of P^+ formation of the nanosecond time-scale is equal to that of *Rb. sphaeroides*, i.e., close to unity. The reported discrepancy to the millisecond data is indeed due to the loss of Q_A in the *Chloroflexus* preparation used in Ref. 8, as already suggested there.

This conclusion rests on the exclusion of the following alternative explanations for the low quantum yield of [8]:

(1) Appreciable losses from $^1P^*$ in *Chloroflexus*, e.g., by internal conversion to the ground state, would not be in agreement with the high quantum yield of P^+ measured on the nanosecond timescale.

(2) Since picosecond measurements exclude a lifetime of a possible state $P^+H_B^-$ in the nanosecond range or longer [18,19], the high quantum yield of P^+ measured after nanoseconds rules out losses by charge separation to the B-branch.

(3) As in the case of *Rb. sphaeroides*, also in RCs of *Chloroflexus* the forward electron transfer from $P^+H_A^-$ to P^+Q^- [19,20] is fast compared to recombination of $P^+H_A^-$ (our unpublished data), excluding losses from $P^+H_A^-$ prior to formation of P^+Q^-.

The determination of the quantum yield is of special relevance for RCs exhibiting slow primary charge separation, as encountered in *Chloroflexus* at low temperatures [16,17] and in specific mutants from *Rb.*

[×] To obtain this result, a value of $\Delta\epsilon_{865} = 112\ mM^{-1}\ cm^{-1}$ [32] was assumed; as discussed in Appendix II, this value could be slightly too small, a higher value leading to a smaller QY. However, a determination of the QY from the light saturation curve, which does not make use of the value of $\Delta\epsilon_{865}$, yielded the very similar result of 0.98 ± 0.04 with excitation at 583.5 nm [35].

sphaeroides [38,39]. In these cases the quantum yield is expected to be significantly lowered in case the internal conversion rate $^1P^* \rightarrow P$ is sufficiently fast. In fact, in the LL-mutant of *Rb. capsulatus* where no primary charge separation is observed, the lifetimes of $^1P^*$ is of the order of only 100 ps [40]. Since, however, in the above-mentioned preparations significant losses of both quinones are observed, quantum yield measurement will have to be performed on the nanosecond timescale.

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Appendix I

Calculation of the averaged excitation probability ϕ^*_{av}

A light pulse with an intensity N (number of photons per unit area per pulse) excites molecules with a cross-section for absorption σ in a thin layer dx of the sample with the excitation probability ϕ^*_N:

$$\phi^*_N = 1 - e^{-\sigma N} \tag{A1}$$

By absorption in the sample, N decreases with the distance x that the light already passed in the sample ($A(x)$ being the absorbance of that part of the sample which the light already passed):

$$N(x) = N_0 10^{-A(x)} \tag{A2}$$

Assuming a homogeneous distribution of molecules in a sample with absorbance A_0 and thickness d yields for $A(x)$:

$$A(x) = \frac{A_0}{d} x \tag{A3}$$

Averaging ϕ^*_N over the sample and inserting Eqns. A1–A3 finally gives:

$$\phi^*_{av} = \frac{1}{d}\int_0^d \phi^*_N(x)\,dx = 1 - \frac{1}{A_0 \ln 10}\int_{10^{-A_0}}^1 \frac{e^{-\sigma N_0 y}}{y}\,dy \tag{A4}$$

Here, the effect of the decrease of A_0 during the exciting laser pulse was ignored, since a total of only 30% of the RCs were excited during the pulse, causing a decrease of the 600 nm absorption of P and BChl monomers of less than about 15%.

Appendix II

Equality of $\Delta\epsilon_{pr}/\epsilon_{pr}$ *in RCs of Rb. sphaeroides and Chloroflexus*

$\Delta\epsilon/\epsilon$ in the Q_y transition of P has been reported to be 0.88 for *Rb. sphaeroides* [32] and 0.9 for *Chloroflexus* [25]. These values, however, could have been determined too small because of a small fraction of RCs having lost both quinones during preparation. Also in Ref. 32 a slightly higher value of 0.92 ± 0.05 was determined for $\Delta\epsilon/\epsilon$ from comparing the bleaching of P with the bleaching of added cytochrome *c* due to oxidation of cytochrome *c* by P^+. This method takes into account only those RCs with an intact electron transfer to Q. Therefore the reported values do not allow the comparison of $\Delta\epsilon/\epsilon$ in the two types of RC with the accuracy needed here.

The absorption spectra of RCs of *Rb. sphaeroides* and *Chloroflexus* are identical at wavelengths over 865 nm, when normalized at 865 nm [9], where there extinction coefficients have been shown to be the same within 2% [34]. These results show the spectral identify of the lower exciton band of the Q_y transition of P around 865 nm in the two RCs. A small residual absorption of the BChl monomer Q_y transition, which is centered near 800 nm, in this spectral region could in principle be different in RCs of *Rb. sphaeroides* and *Chloroflexus*, due to the different numbers of BChl monomers. However, the red shift of the BChl monomer absorption in *Chloroflexus* compared to that in *Rb. sphaeroides* (812 nm compared to 802 nm) [9] seems to compensate for this difference, as can be deduced from the correspondence of extinction coefficient and spectral shape.

Besides possible losses of both quinones, the reported incomplete bleaching of the absorbance at 865 nm could be due either to such a residual absorption of the BChl monomers or to some absorption of the dimer cation P^+, which should be very similar in *Rb. sphaeroides* and *Chloroflexus*. In any case, identical values are expected for $\Delta\epsilon_{pr}/\epsilon_{pr}$ in the two types of RC.

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