Biochimica et Biophysica Acta, 936 (1988) 124–132
Elsevier

BBA 42853

pH effect on the biphasicity of the $P^+Q_A^-$ charge recombination kinetics in the reaction centers from *Rhodobacter sphaeroides*, reconstituted with anthraquinones

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(Received 8 February 1988)

Key words: Bacterial photosynthesis; Reaction center; Recombination kinetics; pH dependence; Protonation; (*Rb. sphaeroides*); (*Rps. viridis*)

The rate constant of decay of the flash-induced absorbance changes related to the primary electron donor, P^+, was measured in anthraquinone-reconstituted reaction centers from wild type *Rhodobacter sphaeroides* (strain Y). The decay was found to be biphasic. At pH 9, the two rate constants are equal to $166 \pm 20\ s^{-1}$ (k_{slow}) and $350 \pm 30\ s^{-1}$ (k_{fast}), and their amplitudes are 55% and 45%, respectively. This apparent biphasicity is strongly pH-dependent. At pH 11.2, both components are accelerated ($k_{slow} = 370 \pm 40\ s^{-1}$ and $k_{fast} = 1440 \pm 100\ s^{-1}$) but their relative amplitudes are inverted to 25% and 75%, respectively The pH dependence curves of both the rate constants and relative amplitudes of the two phases are very similar to what was recently observed in the native reaction centers from *Rhodopseudomonas viridis* (Sebban, P. and Wraight, C.A., unpublished data). The increase in the rate constants above pH 9 reflects a diminution of the free energy difference between the $P^+Q_A^-$ state and a thermally excited state (possibly P^+I^-) via which P^+ and Q_A^- recombine. The pH dependence curves of k_{slow}, k_{fast} or the average rate constant, display a pK value (pK_{Q_A}) of about 10.3, indicating that the replacement of the native ubiquinone by an anthraquinone shifts the pK of protonation of Q_A^- compared to the native ubiquinone (pK = 9.8). The replacement of the native Q_A by the 1-amino-5-chloroanthraquinone or the 1-chloroanthraquinone confirmed this pK_{Q_A} shift. The obtained pK_{Q_A} value is independent of the presence of terbutryn. In addition to these similarities, the activation parameters of k_{slow} and k_{fast} also behave as in *Rps. viridis*. From the Arrhenius plots of the two components, we determined that $\Delta H_{slow} < \Delta H_{fast}$, but because of the quite large entropic contributions, $\Delta G_{slow} = 0.295 \pm 0.01$ eV $> \Delta G_{fast} = 0.276 \pm 0.01$ eV. It is suggested that the observed biphasicity of the charge recombination is due to the fast recombination rate in the anthraquinone-reconstituted *Rb. sphaeroides* reaction centers (as in native reaction centers from *Rps. viridis*), which prevents the different protonation states reached after the flash from equilibrating. This is in contrast to what is observed in native ubiquinone-containing reaction centers where the recombination rate is much slower.

Introduction

The electromagnetic energy absorbed by the antenna of photosynthetic organisms is converted to chemical energy at the level of the reaction centers. The first stable charge separation takes

Abbreviations: CAPS, cyclohexylaminopropanesulfonic acid; LDAO, lauryldimethylamine *N*-oxide.

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place between the primary electron donor (a dimer of bacteriochlorophyll in the case of the purple photosynthetic bacteria), P, and a molecule of quinone, Q_A. The electron present on Q_A is then transferred to a secondary quinone molecule Q_B. When Q_B is chemically extracted, or in the presence of an inhibitor of the Q_A to Q_B electron transfer, the charges on P^+ and Q_A^- will recombine. In the reaction centers from *Rhodobacter sphaeroides*, this recombination occurs in about 100 ms, directly to the ground state. However, it has been shown that, when the native Q_A (ubiquinone 10; UQ_{10}) is replaced by other quinones of lower in vivo redox potential, the rate constant of $P^+Q_A^-$ charge recombination is notably increased. For sufficiently low-potential quinones, the rate constant of recombination is directly dependent on the in vivo redox potential of Q_A [1]. The lower the E_m of the Q_A/Q_A^- couple, the faster the recombination kinetics. This interpreted in terms of the recombination between P^+ and Q_A^- occurring either directly to the ground state PQ_A, or partly via a thermally excited state, M[1–7]. The amount of $P^+Q_A^-$ that repopulates M depends on the free energy difference, ΔG, between $P^+Q_A^-$ and M. It has been proposed that M could be identical to P^+I^-, the first charge-separated state in the forward pathway [2,6,7], where I is a bacteriopheophytin molecule. However, this hypothesis implies a substantial relaxation (≈ 0.2 eV) of the free energy of P^+I^-, in the millisecond time range [1]. In *Rhodopseudomonas viridis*, the $P^+Q_A^-$ recombination kinetics are much faster (≈ 1 ms) than in *Rb. sphaeroides* [7–9]. This probably arises from a smaller energy spacing between $P^+Q_A^-$ and M, compared to *Rb. sphaeroides*. Furthermore, the charge recombination kinetics in the presence of *o*-phenanthroline, or when Q_B is absent, are biphasic in *Rps. viridis*, contrary to *Rb. sphaeroides* (P. Sebban and C.A. Wraight, unpublished data). This was interpreted as due to the kinetic competition between the recombination rate and the establishment of protonation equilibria after the flash, i.e. the protonation and deprotonation rates are presumably competitive with the recombination rate of *Rps. viridis*, but much faster than the recombination rate in *Rb. sphaeroides*.

In order to test this hypothesis concerning the origin of biphasicity in *Rps. viridis*, we replaced the native UQ_{10} in the reaction centers from *Rb. sphaeroides* by an anthraquinone. In such preparations, the rate of charge recombination is similar to that in *Rps. viridis* [1,7]. At variance to what was previously reported for such preparations [2], we find the kinetics to be biphasic. The energetics and the pH dependence behavior of the two components are similar to what we observed in *Rps. viridis*.

Material and Methods

The native reaction centers from the wild type *Rb. sphaeroides*, strain Y, were prepared as previously described by Rivas et al. [10]. To remove the primary ubiquinone (Q_A), the method of Okamura et al. [11] was used with some modifications introduced by Woodbury et al. [1]. The Q_A-depleted reaction centers were eluted from the DEAE-52 Sephacryl (Pharmacia) column with 0.33 M NaCl instead of 0.375 M. The $\Delta A_{280\,nm}/\Delta A_{802\,nm}$ ratio was in the range 1.28–1.32. Judging from the obtained kinetics of charge-recombination, about 95% of the reaction centers were depleted of ubiquinone. The remaining 5% displayed a 116 ms decay lifetime as native reaction centers, in the presence of an inhibitor of Q_A to Q_B electron transfer. In addition, when depleted reaction centers were reconstituted with UQ_{10}, they displayed a mono-exponential decay, in the presence of terbutryn, with a lifetime of 110 ms.

The anthraquinone (from Fluka, 99% pure by HPLC) solution was dissolved in dimethyl sulfoxide. A 10-fold excess was used in the experiments. The final concentration of this solvent was less than 0.5%. 40 μM terbutryn was commonly added to the solution, but no noticeable effect could be ascribed to this inhibitor, suggesting that the Q_B site was not reactivated by anthraquinone. The quantum yield of $P^+Q_A^-$ formation in the anthraquinone-reconstituted reaction centers was 90% of that of UQ_{10}-reconstituted reaction centers.

The absorbance changes were recorded on a home-made flash spectrophotometer described elsewhere [12]. The exponential decomposition analysis of the kinetics was performed as described previously [12]. The standard error on the

pH measurements was 0.05 unit. The temperature measurements were done with a thermocouple with a precision of 0.3°.

Biphasicity of the charge recombination kinetics

The $P^+Q_A^-$ decay kinetics of the anthraquinone-reconstituted reaction centers from *Rb. sphaeroides*, observed at 865 nm and 23°C, are shown in Fig. 1. The average lifetime of the decay is about 5 ms, in the pH range 6–8, in agreement with earlier studies [1–7].

The traces of Fig. 1 were fitted either with two exponentials (a and b) or with three exponentials (c and d). In both cases, one component decays with a 110 ms lifetime and represents about 5% of the total amplitude. It arises from the $P^+Q_A^-$ recombination occurring in the reaction centers that had retained the native Q_A.

When an exponential is used to fit the fast part (95% of the total amplitude) of the decays (Fig. 1a and b), the noise of the data-model curves, represented below the decays, is clearly not random. Two exponentials (at least) are needed to fit the fast part of the decay. At pH 8.6 (Fig. 1c), their lifetimes are 6 ms (k_{slow}^{-1}) and 2.9 ms (k_{fast}^{-1}) and the ratio of their amplitudes 55:45. The result varies strongly with pH. At pH 11.2 (Fig. 1d) both components are faster (2.9 ms and 0.75 ms), and their amplitude ratio has changed to 25:75. It must be noted that the total amplitude of the observed P^+ decay is the same within 10% in both cases, suggesting that both components intercon-

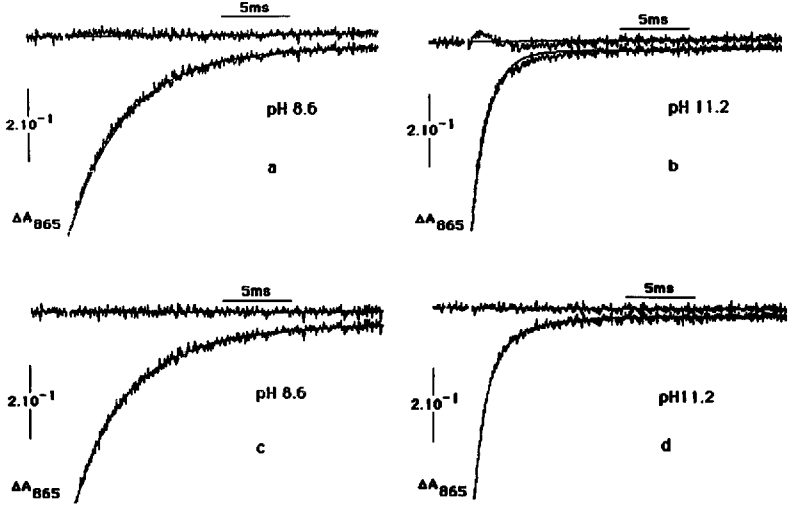


Fig. 1. Charge recombination kinetics, measured at 865 nm and 23°C, in *Rb. sphaeroides* reaction centers where the native ubiquinone, Q_A, is replaced by an anthraquinone. Except for the 5% amplitude due to the remaining native ubiquinone, these decays are fitted either with an exponential (a and b) or by the sum of two exponentials (c and d). At pH 8.6 (c), the two lifetimes are 6 ms and 2.9 ms, with relative amplitudes of 55% and 45%, respectively. At pH 11.2 (d), the lifetimes are 2.9 ms and 0.75 ms, and the amplitudes 25% and 75%, respectively. The total observed amplitude of the P^+ signal is the same in both cases, within 10%. The differences between the experimental and the model curves are shown at the top of the decays. Conditions: at pH 8.6, 20 mM Tris-HCl, 0.1% LDAO, 40 μM terbutryn; at pH 11.2, idem except 20 mM CAPS, in place of Tris.

vert as the pH changes. The same decompositions were obtained at 430 nm and 600 nm.

These data are not in agreement with those of Kleinfeld et al. [6]. These authors reported a single component for the $P^+Q_A^-$ decay from the R26 reaction centers from *Rb. sphaeroides*, reconstituted with anthraquinone. We do not think that our results are due to some type of heterogeneity of the reaction centers preparation since the UQ_{10}-reconstituted reaction centers gave a mono-

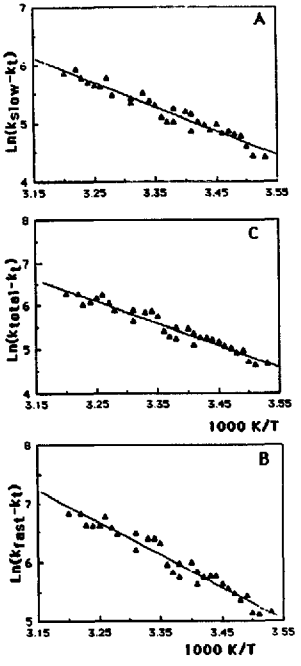


Fig. 2. Arrhenius plots of the rate constants for charge recombination in anthraquinone-reconstituted reaction centers from *Rb. sphaeroides*. For the reasons described in the text, k_T was taken as 10 s^{-1}. Conditions: 20 mM Tris-HCl (pH 9), 0.1% LDAO, 40 μM terbutryn.

exponential $P^+Q_A^-$ decay. We exclude too the presence of an impurity in our anthraquinone solution since the relative amplitudes of k_{slow} and k_{fast} vary as the pH increases (see below) in a very similar way to that observed in the native reaction centers from *Rps. viridis* (P. Sebban and C.A. Wraight, unpublished data).

Temperature dependence of both components

The Arrhenius plots of k_{slow} and k_{fast} measured at pH 9 are shown on Fig. 2. The low temperature dependence (down to 80 K) of the rate constant of the average decay kinetics of anthraquinone-reconstituted reaction centers from *Rb. sphaeroides* has been recently measured by Shopes and Wraight [7]. These authors found that the behavior of these reaction centers was similar to that of the native reaction centers from *Rps. viridis*. It was postulated that, in both cases, the charge recombination process occurs by two competitive pathways: a direct electron-tunneling process that dominates at low (< 250 K) temperature and a thermally activated process via an energetically elevated state, M, at higher temperature. This was described by the equation:

$$k_{total} = k_d \cdot \exp(-\Delta G/kT) + k_T \quad (1)$$

where ΔG represents the free energy difference between the $P^+Q_A^-$ state and M. k_{total} represents the inverse of the average lifetime of the fast part of the decay. k_T is the limiting value of k_{total}, measured at temperatures below 200 K. k_d is the rate constant of decay from this state to the ground state. If one assumes that M is P^+I^-, then k_d represents the rate constant of P^+I^- charge recombination. Similarly, Sebban and Wraight (unpublished data) proposed for both components observed in *Rps. viridis*:

$$k_{slow} = k_d \cdot \exp(-\Delta G_{slow}/kT) + k_{T\,slow}$$

$$k_{fast} = k_d \cdot \exp(-\Delta G_{fast}/kT) + k_{T\,fast} \quad (2)$$

with corresponding definitions as for k_{total}.

Consequently, in the Arrhenius plots, we have subtracted from k_{slow} and k_{fast} the limiting value of the rate constant measured at low temperature by Shopes and Wraight [7]. This can only be an

TABLE I

ACTIVATION PARAMETERS FOR THE $P^+ Q_A^-$ CHARGE RECOMBINATION IN ANTHRAQUINONE-RECONSTITUTED REACTION CENTERS FROM *RB. SPHAEROIDES*

Conditions: 20 mM Tris-HCl (pH 9), 0.1% LDAO, 40 μM terbutryn, $T = 295$ K.

	k_t (s^{-1})	ΔH (eV)	ΔS (meV/degree)	$-T\Delta S$ (eV)	ΔG (eV)
k_{slow}	10	0.350 (±0.025)	0.185 (±0.05)	−0.055 (±0.015)	0.295 (±0.01)
k_{fast}	10	0.439 (±0.025)	0.551 (±0.06)	−0.163 (±0.016)	0.276 (±0.01)
k_{total}	10	0.413 (±0.025)	0.412 (±0.05)	−0.122 (±0.02)	0.291 (±0.01)

approximate solution because we subtracted the same value (k_T) for k_{slow} and k_{fast} (instead of $k_{T\,slow}$ and $k_{T\,fast}$), and also because this value was obtained in glycerol instead of aqueous solvent, as we used here. But in fact, because the difference in enthalpy (ΔH) measured in anthraquinone-reconstituted reaction centers is very large, the limiting value of the rate constant, measured at low temperature, is almost negligible compared to its value at room temperature. Thus, the results are rather insensitive to the small correction we have used.

The thermodynamic parameters derived from the plots of Fig. 2 are shown in Table I. At pH 9, ΔH_{slow}, ΔH_{fast} and ΔH_{total} are found to be 0.35 ± 0.025 eV, 0.439 ± 0.025 eV and 0.413 ± 0.025 eV, respectively. ΔH_{slow} and ΔH_{fast} are substantially higher than those found for k_{slow} and k_{fast} in *Rps. viridis*. However, similarly to what is observed for *Rps. viridis*, the entropic contributions (obtained from the intercept of the line with the Y axis) are not negligible (Table I). They both make negative contributions to the ΔG values. It is interesting to note that, $\Delta H_{slow} < \Delta H_{fast}$, but $\Delta G_{slow} > \Delta G_{fast}$. At 295 K, we obtain $\Delta G_{slow} = 0.295 \pm 0.01$ eV and $\Delta G_{fast} = 0.276 \pm 0.01$ eV ($\Delta G_{total} = 0.291 \pm 0.01$ eV). According to Eqns. 2, this 21 meV difference should imply a k_{fast}/k_{slow} ratio of 2.3. We obtain at pH 9 a value of about 2.1.

The ΔH_{total} value (0.413 ± 0.025 eV) measured at pH 9 is in reasonable agreement with the data for anthraquinone-reconstituted reaction centers in 60% glycerol at pH 7, $\Delta H = 0.47 \pm 0.02$ eV [7], in aqueous buffer at pH 8, 0.37 ± 0.02 eV [1] and in lipid bilayers, 0.43 ± 0.02 eV [2].

The choice of k_d is important in the determination of the entropic contribution to the ΔG. We took here a value of $2 \cdot 10^7$ s^{-1} as discussed by Shopes and Wraight [7] and by Woodbury et al. [1]. The decay rate of P^+I^- in the reaction centers from *Rb. sphaeroides*, with Q_A extracted was estimated to be $k = 7 \cdot 10^7$ s^{-1} [13–15]. However, in open reaction centers, when the $P^+Q_A^-$ state is allowed to be populated, rapid mixing of $^1[P^+Q_A^-]$ and $^3[P^+Q_A^-]$ occurs because of the fast spin coupling between the electron on Q_A^- and the iron atom in the $S = 2$ state. As a consequence, the same spin distribution as that induced between $^1[P^+Q_A^-]$ and $^3[P^+Q_A^-]$ (25 : 75) is generated by back electron transfer between $^1[P^+I^-]$ and $^3[P^+I^-]$. For these reasons, and because the return to the ground state from P^F occurs mainly via the singlet channel [14], i.e. from $^1[P^+I^-]$, the decay rate of the P^F state reached after charge recombination between P^+ and Q_A^- is necessarily different from k, and may be taken as $k/4$, i.e. $2 \cdot 10^7$ s^{-1} [7]. The above ΔG values were obtained assuming this value. Taking $k_d = 7 \cdot 10^7$ s^{-1} would lead to $\Delta G_{total} = 0.322$ eV, instead of 0.291 eV. The former value is in excellent agreement with the results of Gunner et al. [4] (0.326 eV), but we rather think that the lower figure obtained with $k_d = 2 \cdot 10^7$ s^{-1} should be used. However, one could object that if the $P^+Q_A^-$ state effectively recombines as described above, a k_d value closer to k_t, the rate constant of recombination from P^+I^- via the triplet channel, should be used here. If we taken $k_d = 5 \cdot 10^8$ s^{-1}, the k_t value suggested by Norris et al. [16], we find $\Delta G_{total} = 0.37$ eV. Even with such a substantial ΔG_{total} difference (0.08 eV), our conclusions of the next paragraph about the possible relaxation of the P^F state in the ms time range are not much changed.

By measuring the delayed fluorescence of anthraquinone-reconstituted reaction centers from R26, Woodbury et al. [1] estimated the free energy difference between P^* and $P^+Q_A^-$ to be 0.7 eV at room temperature. If 0.29 eV (obtained here for ΔG_{total}) is subtracted from this value, we find that M should be about 0.4 eV below P^*, in free

energy. The above authors found 0.188 eV free energy difference between P* and the most relaxed state of P^F, since it was previously suggested that this state is stabilized (in the ns time range) during its lifetime [17–20]. To identify M with P^F, one must then assume that P^F can relax by about 0.22 eV, in the ms time range. Even in the limiting case where $k_d = k_t = 5 \cdot 10^8\ s^{-1}$, which leads to $\Delta G_{total} = 0.37$ eV (see above), a 0.14 eV relaxation of P^F is still needed to identify M with P^F. The same hypothesis had to be proposed for the native reaction centers from *Rps. viridis* [7]. However, in that case the amplitude of the putative relaxation

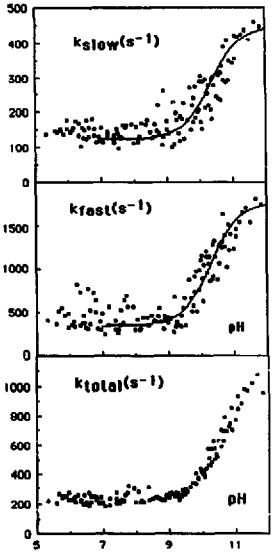


Fig. 3. pH dependence of k_{slow}, k_{fast} and k_{total} observed at 865 nm and 23°C in anthraquinone-reconstituted reaction centers from *Rb. sphaeroides*, in the presence of 40 μM terbutryn. The lines are drawn according to Eqn. 3 with $k_{slow}^{H^+}$ and k_{slow}^{0} equal to 125 s^{-1} and 448 s^{-1}, respectively, $k_{fast}^{H^+}$ and k_{fast}^{0}, 345 s^{-1} and 1770 s^{-1}, respectively. With these parameters, the best fit is obtained for $pK_{QA} = 10.3$ for k_{slow}, and $pK_{QA} = 10.35$ for k_{fast}.

of P^F (in the ms time range) is much smaller if not negligible, since M was proposed to be only 0.3 eV (and even less if k_d is taken equal to k_t and not $2 \cdot 10^7\ s^{-1}$) below P*, and P^F was found to be 0.25 eV below P* in free energy [21]. Thus, if M is a relaxed form of P^F, the amplitude of the relaxation of this state in the long time range appears to be very different in *Rb. sphaeroides* compared to *Rps. viridis*.

pH dependence of k_{slow} and k_{fast}

The pH dependence curves of k_{slow}, k_{fast} and k_{total} are shown in Fig. 3. According to Eqns. 2, any change in the ΔG_i affects the corresponding k_i values. Thus, any variation in the k_i values can be understood in terms of the interaction of a proton with Q_A^-, that modifies the ΔG_i, as was previously suggested [6]. To fit the curves of Fig. 3 we used the equation:

$$k_i = \frac{k_i^0 + 10^{(pK_{Q_A} - pH)} \cdot k_i^{H^+}}{1 + 10^{(pK_{Q_A} - pH)}} \qquad (3)$$

where i denotes total, slow or fast. k_i^0 and $k_i^{H^+}$ are the rate constants of charge recombination measured in the unprotonated or protonated form of the reaction centers. pK_{QA} is the pK of protonation of a site near Q_A^-. From the fit of Fig. 3, we find that pK_{QA} is about 10.3–10.4 for k_{slow} and k_{fast} (and also for k_{total}). The absence of terbutryn doesn't shift this pK to lower pH, at variance to what is commonly observed for *o*-phenanthroline in *Rps. viridis* reaction centers [22]. The above pK_{QA} figure disagrees with previous data [6] which supported a pK_{QA} value of 9.8 in anthraquinone-reconstituted reaction centers from *Rb. sphaeroides*, unchanged compared to the native reaction centers. However, we also found (P. Sebban, unpublished data) a pK_{QA} value of 10.3 in the cases of the 1-amino-5-chloro-anthraquinone- and 1-chloro-anthraquinone-reconstituted reaction centers from *Rb. sphaeroides*. This change in the pK of protonation of Q_A^- is not really surprising. A 0.5 pH unit shift in the pK_{QA} value is equivalent to an electrostatic interaction energy change of about 0.029 eV between Q_A^- and a proton that probably binds to a nearby amino-acid residue. Kleinfeld et al. [6] have suggested that the distance (d) between Q_A^- and the protonation site could be

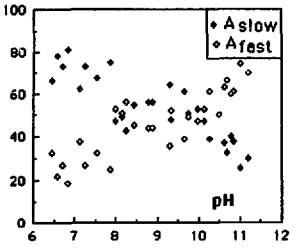
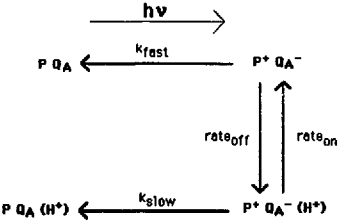


Fig. 4. pH dependence of A_{slow} (◆) and A_{fast} (◇), the relative amplitudes of k_{slow} and k_{fast}, measured at 865 nm, in anthraquinone-reconstituted reaction centers from *Rb. sphaeroides*. Conditions: 0.1% LDAO, 40 μM terbutryn.



Scheme I. Scheme representing the competition between the recombination to the ground state and the establishment of equilibrium between protonated and unprotonated forms of $P^+Q_A^-$. k_{slow}, k_{fast}, $rate_{on}$ and $rate_{off}$ are defined in the text.

≥ 5 Å. Taking $d = 6$ Å leads to a change of 0.45 Å ($d = 7$ Å leads to 0.6 Å) in the distance from the negative charge present on Q_A^- to the protonation site, if the native ubiquinone is replaced by an anthraquinone. Replacing the native ubiquinone by an anthraquinone probably extends the delocalization of the negative charge to the three conjugated rings and may be responsible for the observed distance change and pK_{QA} shift.

From the magnitude of the increases in the rate constants between pH < 8.5 and pH > 11, one can estimate the interaction energy (changes in the ΔG) due to the effect of protonation: $\Delta\Delta G_{slow} = 0.034 \pm 0.03$ eV, $\Delta\Delta G_{fast} = 0.040 \pm 0.04$ eV.

The pH dependence curves of the relative amplitudes of the slow (A_{slow}) and the fast phase (A_{fast}) are shown in Fig. 4. They are very similar to what was observed in the reaction centers from *Rps. viridis*. These curves display two waves, separated by a plateau from pH 8 to pH 10. Below pH 8, A_{slow} dominates. Between pH 8 and pH 10, both amplitudes stay roughly constant, but at high pH, A_{fast} becomes the main component. For electrostatic reasons, it is reasonable to assume that A_{fast} is proportional to the number of reaction centers that recombine via the $P^+Q_A^-$ state instead of the protonated state $P^+Q_A^-(H^+)$, the former state being more and more populated as the pH increases. In support of this hypothesis, the total amplitude of the P^+ signal is constant within 10% up to pH 11.4, suggesting that we observe an interconversion of the amplitudes of both components, as the pH increases, instead of the degradation of a part of the reaction center preparation. These ideas are worked out more fully in a model that will be presented elsewhere (P. Sebban and C.A. Wraight, unpublished data), on the basis of results obtained on *Rps. viridis* reaction centers.

Discussion

Maróti and Wraight [23] and Maróti (unpublished data) have suggested that the molar rate constant (k_{on}) of proton binding to the reaction centers could be as high as 10^{12}–10^{13} $M^{-1} \cdot s^{-1}$. The establishment of equilibrium between the protonated and unprotonated forms of $P^+Q_A^-$ depends on the magnitude of the term ($rate_{on}$ + $rate_{off}$) compared to the decay rate constant to the ground state (i.e. k_{slow} and k_{fast}) (see Scheme I). $rate_{on}$ and $rate_{off}$ are the first-order rate constants of proton uptake and release to the Q_A^- protonation site, respectively. $rate_{on}$ is equal to $k_{on} \cdot 10^{-pH}$, and $rate_{off}$ which is pH-independent, equals $rate_{on}$ at $pH = pK_{QA}$. If we take $k_{on} \geq 10^{12}$ $M^{-1} \cdot s^{-1}$, and $pK_{QA} = 9.8$ as in native *Rb. sphaeroides* reaction centers, then $rate_{off} \geq 10^{2.2}$ $s^{-1} \approx 160$ s^{-1}.

At pH < 10, $(rate_{on} + rate_{off}) \geq 10^{12} \cdot 10^{-10} + 160 \approx 100 + 160 \approx 260$ s^{-1}. This value is much greater than the rate constant of charge recombination (10 s^{-1}) observed in the native reaction centers from *Rb. sphaeroides*. The observed $P^+Q_A^-$ decay is then exponential. At pH ≥ 11, $rate_{on}$ becomes negligible, but $rate_{off}$ is still ≥ 160 s^{-1},

which maintains the monophasicity of the measured decay.

In *Rps. viridis*, or in *Rb. sphaeroides* where Q_A has been replaced by a low-potential quinone, the rate constants of charge recombination are much higher (200–1000 s^{-1}) and compete with ($rate_{on}$ + $rate_{off}$) at pH ≥ 9. Thus, the equilibrium between the protonation states, reached after the flash, cannot be established and the $P^+Q_A^-$ decay is biphasic. At pH ≤ 7, ($rate_{on}$ + $rate_{off}$) ≥ $10^{12} \cdot 10^{-7}$ = 10^5 $s^{-1} \gg 1000$ s^{-1}. The observed $P^+Q_A^-$ decay is then exponential. This is shown in Fig. 4 where A_{fast} becomes smaller than 20% at pH ≤ 7.

Parot et al. [24] have recently detected a biphasicity of the charge recombination kinetics from $P^+Q_A^-$ in native reaction centers from *Rb. sphaeroides* and *Rhodospirillum rubrum* at low temperature. These kinetics exhibited lifetimes of 36 ms and 11 ms. The ratio of these two components was 40 : 60, respectively. However, there are two significant differences compared to the biphasicity that we observe here or in the native reaction centers from *Rps. viridis*. First, the kinetics in *Rb. sphaeroides* and *R. rubrum* were biphasic only at low temperature. Second, the amplitude ratio of the two components was not sensitive to pH. The relationship between the observations of Parot et al. and the present work is obscure. It is not sufficient to suggest that the biphasicity, in *Rb. sphaeroides* and *R. rubrum* were biphasic only at low temperature. Second, the amplitude ratio of the two components was not sensitive to pH. The relationship between the observations of Parot et al. and the present work is obscure. It is not sufficient to suggest that the biphasicity, in *Rb. sphaeroides* is observable at low temperature because the kinetics accelerate enough to be in competition with $rate_{on}$ and $rate_{off}$. One would still expect to observe a pH sensitivity of the relative amplitudes of the two components, unless this arises outside the range observed by these authors – pH 6–9 – or if there were some remarkable temperature compensation of the pH effect.

Conclusion

It was recently shown that the kinetics of $P^+Q_A^-$ decay are biphasic , at room temperature, in reaction centers of *Rps. viridis*, in contrast to what is observed in reaction centers of *Rb. sphaeroides*. It was suggested that this reflects non-equilibrium between distinct protonated states of $P^+Q_A^-$, after a flash. At variance to *Rb. sphaeroides*, where the rate constant of charge recombination is slow, the fast rate observed in *Rps. viridis* competes with the establishment of protonation equilibrium. In order to check this hypothesis, we replaced in the reaction centers of *Rb. sphaeroides* the native UQ_{10} by an anthraquinone which has a lower in vivo midpoint redox potential. The $P^+Q_A^-$ recombination decay is then accelerated to a rate close to what is observed in the native reaction centers of *Rps. viridis*. In these modified preparations, we observe the same type of biphasicity of the $P^+Q_A^-$ decay as in *Rps. viridis*, varying in a very similar way as a function of pH. In addition, we observe the same relationship between the thermodynamic activation parameters related to k_{slow} and k_{fast}. For these reasons, we think that we observe here the same phenomenon as previously in the native reaction centers from *Rps. viridis*.

Acknowledgements

I thank C.A. Wraight and B.R. Velthuys for helpful discussions and careful reading of the manuscript. F. Reiss-Husson and I. Agalidis are also acknowledged for their suggestions. I thank J.C. Barbet for his good job on the exponential analysis program.

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