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Primary donor recovery kinetics in reaction centers from *Rhodopseudomonas viridis*. The influence of ferricyanide as a rapid oxidant of the acceptor quinones

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In reaction centers from *Rhodopseudomonas viridis* that contain a single quinone, the decay of the photo-oxidized primary donor, P^+, was found to be biphasic when the bound, donor cytochromes were chemically oxidized by ferricyanide. The ratio of the two phases was dependent on pH with an apparent p*K* of 7.6. A fast phase, which dominated at high pH ($t_{1/2} = 1$ ms at pH 9.5), corresponded to the expected charge recombination of P^+ and the primary acceptor Q_A^-. A much slower phase dominated at low pH and was shown to arise from a slow reduction of P^+ by ferrocyanide in reaction centers where Q_A^- has been rapidly oxidized by ferricyanide. The rate of Q_A^- oxidation was linear with respect to ferricyanide activity and was strongly pH-dependent. The second-order rate constant, corrected for the activity coefficient of ferricyanide, approached a maximum of $2 \cdot 10^8$ $M^{-1} \cdot s^{-1}$ at low pH, but decreased steadily as the pH was raised above a p*K* of 5.8, indicating that a protonated state of the reaction center was involved. The slow reduction of P^+ by ferrocyanide was also second-order, with a maximum rate constant at low pH of $8 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ corrected for the activity coefficient of ferrocyanide. This rate also decreased at higher pH, with a p*K* of 7.4, indicating that ferrocyanide also was most reactive with a protonated form of the reaction center. The oxidation of Q_A^- by ferricyanide was unaffected by the presence of *o*-phenanthroline, implying that access to Q_A^- was not via the Q_B-binding site. In reaction centers supplemented with ubiquinone, oxidation of reduced secondary quinone, Q_B^-, by ferricyanide was observed but was substantially slower than that for Q_A^-. It is suggested that Q_B^- may be oxidized via Q_A so that the rate is modulated by the equilibrium constant for $Q_A^- Q_B \leftrightarrow Q_A Q_B^-$.

Introduction

The reaction center protein of the purple, non-sulfur photosynthetic bacterium, *Rhodopseudomonas viridis*, consists of four subunits [1]. Electrophoretic analysis [1] and recent X-ray crystallographic studies [2] have suggested three of these to be analogous to the L, M and H subunits of the functionally better characterized *Rhodopseudomonas sphaeroides* reaction center [3]. These subunits bind the bacteriochlorophyll (Bchl) and bacteriopheophytin pigments, active in the earliest photochemical events, and the primary (Q_A) and secondary (Q_B) quinones involved in stabilizing the initial charge separation. In spite of the involvement of BChl *b* in *Rps. viridis*, rather than the much more commonly encountered BChl *a*, the physicochemical properties of the primary donor (P) and the acceptor components are similar to those of other purple bacteria studied so far.

The fourth and largest subunit (40 kDa) of the

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Abbreviation: Cyt, cytochrome.

Rps. viridis reaction center contains four bound, *c*-type hemes: two low-potential cytochromes and two high-potential cytochromes [4]. Both the high- and low-potential cytochromes are capable of submicrosecond donation to P^+. However, under ambient redox potentials, the low-potential cytochromes are oxidized and only the high-potential cytochromes *c*-558 are active as donors.

In the absence of donation to P^+, the various light-induced charge separation states can decay by recombination. The kinetics of the $P^+Q_A^-$ recombination in *Rps. viridis* have been largely studied in chromatophores by the indirect technique of delayed fluorescence. Using three flashes in rapid succession, to oxidize the two high-potential Cyt *c*-558 and set up the state $P^+Q_A^-$, Fleischman and co-workers [5] observed a main decay route of the delayed fluorescence with a rate of 10^3 s^{-1}, but reported other rates of decay as well. Using chromatophores poised at high redox potential with ferricyanide, Carithers and Parson [6] detected delayed fluorescence and also monitored P^+ directly at 960 nm in the presence of *o*-phenanthroline to inhibit forward electron transfer from Q_A. A multiphasic decay of P^+, spanning three decades of time, was observed, accompanied by an unexplained pH dependence. They concluded that the main route of decay was an activation-less tunneling recombination of $P^+Q_A^-$ with a rate of 10^3 s^{-1}. We have previously reported the kinetics of P^+ decay in isolated reaction centers, with the cytochromes fully oxidized but in the absence of ferricyanide. In reaction centers with a single quinone, the decay of P^+ was monophasic and was attributed to the charge recombination of $P^+Q_A^-$, with a rate of $0.63\ (\pm 0.12) \cdot 10^3\ s^{-1}$ at pH 9 [7].

We report here a re-examination of the seemingly complex multiphasic decay of the $P^+Q_A^-$ state in the presence of ferricyanide. The multiphasic kinetics and their pH dependence are understandable when protonation equilibria of the reaction center and interference by exogenous redox mediators are taken into account.

Materials and Methods

Reaction centers and chromatophores were prepared as described by Prince et al. [8]. The ratio of A_{280}/A_{830} was routinely 2.0–2.4 for the reaction centers. Reaction center concentrations were determined using $\Delta\varepsilon^{960\ nm}_{red-ox} = 123\ mM^{-1} \cdot cm^{-1}$ [4]. For most of the experiments, secondary acceptor quinone was extracted from the reaction centers by the method of Okamura et al. [9]. Reaction centers so treated are referred to as Q_B-less. All kinetic experiments were performed as described earlier at room temperature (22–24°C) under anaerobic conditions, with continuous monitoring of pH and redox potential (E_h) [7].

Results

Kinetics of P^+ reduction

The decay kinetics of the pair $P^+Q_A^-$, after a short flash, were studied at high redox potential ($E_h = +440$–450 mV), where the bound, donor cytochromes were oxidized and the primary donor was substantially reduced. Secondary electron transfer to Q_B was eliminated either by quantitative removal of secondary quinone or by the addition of *o*-phenanthroline (2–4 mM).

When the bound cytochrome was oxidized by ferricyanide, two distinct phases of P^+ disappearance were observed: a fast phase, which was completed in about 10 ms, and a considerably slower phase. The relative amounts of the phases were strongly dependent on the pH (Fig. 1). The fraction of the fast phase titrated with a single apparent pK of 7.6 (Fig. 2). Similar behavior could be observed in *Rps. viridis* chromatophores but, in fresh, intact preparations, the kinetics were always dominated by the fast phase. In aged preparations, stored at 4°C for a few days, or in sonicated, fresh chromatophores, the slow phase could be substantial, but appeared at lower pH values than required for isolated reaction centers (apparent p$K < 6.0$). A biphasic P^+ decay could also be induced in Q_B-less *Rps. sphaeroides* R-26 reaction centers under similar conditions (as in Fig. 1), except that the apparent pK was lower (approx. 6.2) (data not shown).

The rate of the fast phase ($t_{1/2} = 1$–2 ms) was consistent with the direct recombination of $P^+Q_A^-$, as described in earlier studies of reaction centers [7] and chromatophores [5,6]. The rate of the slow phase of P^+ decay was linearly dependent on the ferrocyanide activity at constant pH and redox

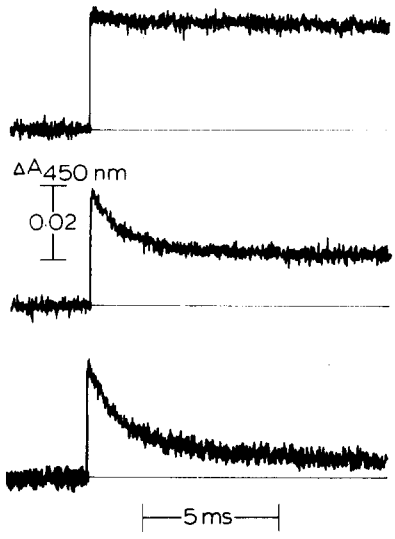


Fig. 1. Decay kinetics of P^+ in the presence of ferricyanide. Kinetics at 450 nm; top to bottom: pH 6.3, 7.8 and 8.8; 1.3 μM reaction centers, 0.1% Triton X-100, 2 mM *o*-phenanthroline, 300 μM ferricyanide, $E_h = +450$ mv.

potential; the large negative charge density on the ferrocyanide anion necessitated a substantial correction for the activity coefficient (Fig. 3A). The slow rate was also linearly dependent on P^+ concentration (data not shown), consistent with a second-order reaction between P^+ and ferrocyanide. The apparent rate constant for the reduction of P^+ by ferrocyanide decreased steadily as the pH was raised (Fig. 3B). A good fit to these

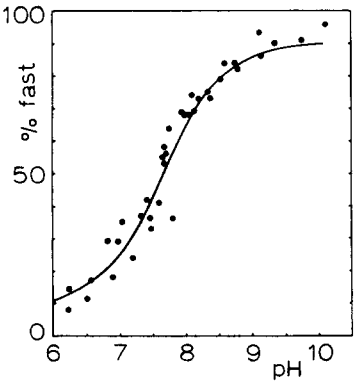


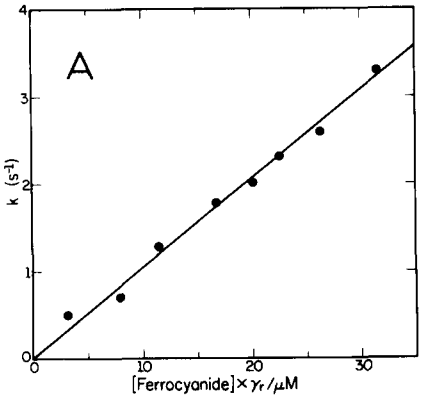
Fig. 2. Dependence on pH of the percentage of P^+ that has decayed 13 ms after the flash (% fast). Conditions as for Fig. 1. The curve is drawn as described in the text.

data is obtained if one assumes pH-independent behavior at low pH, with a maximum rate constant (k_r') of $8 \cdot 10^6$ $M^{-1} \cdot s^{-1}$, and the onset of pH dependence at higher pH, with $pK = 7.4$. The curvature at high pH is not necessarily due to a second pK, but probably indicates the approach to the rate of donation by ferrocyanide to P^+ in an unprotonated reaction center ($k_r = 8 \cdot 10^4$ $M^{-1} \cdot s^{-1}$). The increased scatter at high pH is due to the small amplitude of the slow phase in this pH range (see Fig. 2).

Kinetics of Q_A^- re-oxidation

The decay of P^+ by a slow process, (e.g., reduction by ferrocyanide) can only occur if the recombination pair, $P^+Q_A^-$, has somehow lost its reactivity and does not recombine on its normal time scale of a few milliseconds. This could arise, most simply, if Q_A^- were rapidly oxidized by ferricyanide. The decay of Q_A^- was monitored by several methods. At low redox potential, with 2 mM diaminodurene present, flash-induced Cyt *c*-558^+ was completely re-reduced in less than 0.5 s and the absorption change at 415 nm after this time was due to Q_A^-. In the presence of ferricyanide, with an excess of ferrocyanide to maintain the $E_h = +270$ mV and Cyt *c*-558 90% reduced, the Q_A^- signal could no longer be observed 0.5 s after the flash. When the pH was raised, the flash-induced absorption change at 0.5 s due to Q_A^- returned. In the absence of ferricyanide, the amplitude of absorption due to Q_A^- was pH-independent [7]. These results suggest a rapid oxidation of Q_A^- by ferricyanide at low pH, but not at high pH.

The kinetics of Q_A^- oxidation by ferricyanide could be resolved using a double-flash method. In the absence of ferricyanide (Fig. 4, right-hand curve), the recovery of second-flash activity was due to a slow recombination of Q_A^- with Cyt *c*-558^+ [7]. When ferricyanide was added (with a large excess of ferrocyanide), the ability of a second flash to oxidize Cyt *c*-558 recovered much more rapidly (Fig. 4, left-hand curves). The recovery half-time, equivalent to the re-oxidation half-time of Q_A^-, was 5 ms with 20 μM ferricyanide at pH 6. Raising the concentration of ferricyanide to 50 μM increased the rate of recovery about 2-fold. However, the need for excess



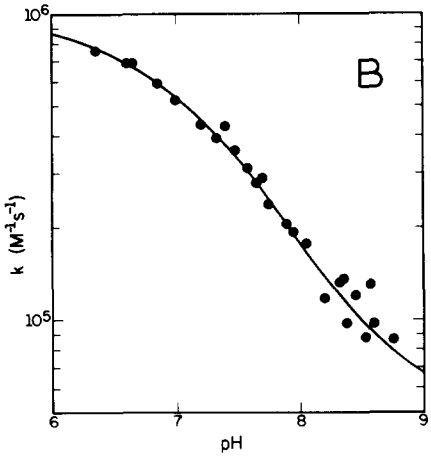


Fig. 3. (A) Dependence of the rate of slow P^+ decay on ferrocyanide activity. 1 μM reaction centers, 2 mM *o*-phenanthroline, 0.1% Triton X-100, 0.5 mM Tris (pH 8.5), $E_h = +453$ mV. Ferrocyanide was added in a 6:94 ratio with ferricyanide, in order to keep the E_h constant. The ferrocyanide concentration was corrected for changes in the activity coefficient, γ_r. The activity coefficients, γ_0 for ferricyanide and γ_r for ferrocyanide, were calculated from:

$$-\log \gamma = Z^2\left\{\frac{0.51\sqrt{I}}{1+0.33a\sqrt{I}} - 0.2I\right\}$$

as given in Ref. 21, with the ionic radius $a = 0.45$ nm, $Z = -3$ (ferricyanide) or -4 (ferrocyanide), and I = ionic strength. (B) Dependence on pH of the second-order rate constant for the reduction of P^+ by ferrocyanide. The second-order rate constant was calculated from the observed half-time from the equation given in Ref. 22:

$$k' = \frac{1}{t_{1/2}}\frac{1}{(A+B)}\ln\frac{2A-B}{A}, \text{ for } A > B,$$

where A is the concentration of ferrocyanide (after correction for its activity coefficient as in A) and B is the reaction center concentration. The curve is drawn, as described in the text, according to:

$$k_r^{obs} = k_r'\frac{10^{pK-pH}}{1+10^{pK-pH}} + k_r\frac{1}{1+10^{pK-pH}}$$

with $k_r' = 9\cdot10^5\ M^{-1}\cdot s^{-1}$, $k_r = 5\cdot10^4\ M^{-1}\cdot s^{-1}$ and $pK = 7.4$.

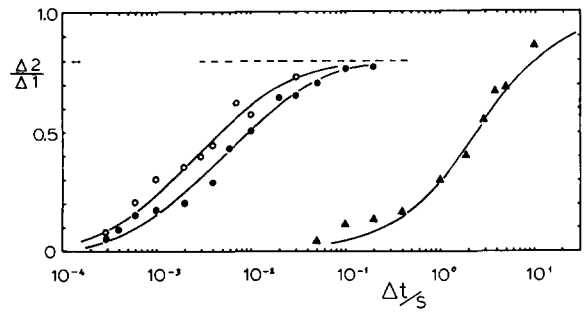


Fig. 4. The rate of Q_A^- oxidation as measured by the amount of Cyt *c*-558 oxidized by the second of two flashes separated by a variable dark time, Δt. 1.3 μM reaction centers, 100 mM NaCl, 0.4 mM *o*-phenanthroline, 0.03% lauryldimethylamine *N*-oxide, 5 mM Tris (pH 6.1). From right to left: (▲) 10 μM ascorbate, 20 μM ferrocyanide, $E_h = +250\pm10$ mV; (●) 3 mM ferrocyanide, 20 μM ferricyanide, $E_h = +280\pm10$ mV; (○) 10 mM ferrocyanide, 50 μM ferricyanide, $E_h = +280\pm10$ mV. The two curves to the left have a lower maximum value, because Cyt *c*-558 is partially oxidized due to the higher E_h.

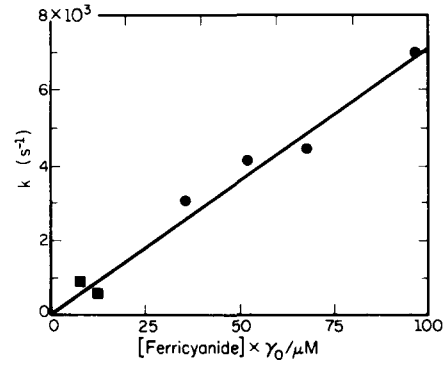


Fig. 5. Oxidation of Q_A^- by ferricyanide. Dependence of the observed rate of Q_A^- oxidation on the ferricyanide activity. Correction for γ_0 as in Fig. 3A. (●) 2.2 μM reaction centers, 2 mM *o*-phenanthroline, 0.1% Triton X-100, 7 mM NaCl, E_h = +440–470 mV, 1 mM Tris (pH 6.2); Q_A^- decay measured at P^+ isosbestic at 422 nm, (■) same but with 0.5 mM ferrocyanide, $E_h = +290\pm310$ mV and Q_A^- decay measured at Cyt *c*-588 isosbestic at 410 nm.

ferrocyanide, in order to keep the cytochromes substantially reduced, caused a significant change in ionic strength. This interfered with a simple linear response of the rate to the added ferricyanide concentration via an effect on the activity coefficient. The dependence of the rate on the ferricyanide concentration was readily resolved at high E_h (+440 mV) by monitoring Q_A^- directly at 422 nm, an isosbestic wavelength for P^+. After correction for the activity coefficient of the ferricyanide (γ_0), the data showed a good linear fit, as expected for a second-order reaction (Fig. 5).

More extensive data on Q_A^- oxidation were obtained at low E_h (+290 mV), measuring Q_A^- directly at 410 nm, an isosbestic for Cyt c-558^+ (Fig. 6A). This method allowed half-times greater than 1 ms to be observed, and the pH dependence of the rate of Q_A^- oxidation was measured by this method (Fig. 6B). At low pH (less than 6), the second-order rate constant, after correction for γ_0, approached a value (k_0') in excess of $2 \cdot 10^8$ $M^{-1} \cdot s^{-1}$, close to the diffusion-controlled limit. At higher pH, above an apparent pK of 5.8, the rate of reaction decreased about one decade per unit increase in pH (Fig. 6B). Above pH 9, the corrected rate constant leveled-off to about $2 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ (k_0). This, in all likelihood, reflects the limiting reactivity of ferricyanide with Q_A^- in deprotonated reaction centers.

The data of Fig. 6B were obtained in the presence of *o*-phenanthroline, but the same behavior was observed for Q_B-less reaction centers in the absence of *o*-phenanthroline (data not shown). It seems, therefore, that ferricyanide does not oxidize Q_A^- via the *o*-phenanthroline-binding site of the reaction center. Furthermore, in reaction centers supplemented with ubiquinone to reconstitute Q_B activity, Q_B^- was much more slowly oxidized by ferricyanide than was Q_A^-, exhibiting a second-order rate constant of about $2 \cdot 10^6$ $M^{-1} \cdot s^{-1}$ at pH 6 (data not shown).

Qualitatively similar behavior was observed with reaction centers from *Rps. sphaeroides*. However, in Q_B-less reaction centers the rate of Q_A^- oxidation by ferricyanide was slower than for *Rps. viridis*. At pH 6, the second-order rate constant was about $4 \cdot 10^5$ $M^{-1} \cdot s^{-1}$. Ferricyanide also oxidized Q_B^- in *Rps. sphaeroides*, but this process was very slow, with a second-order rate constant

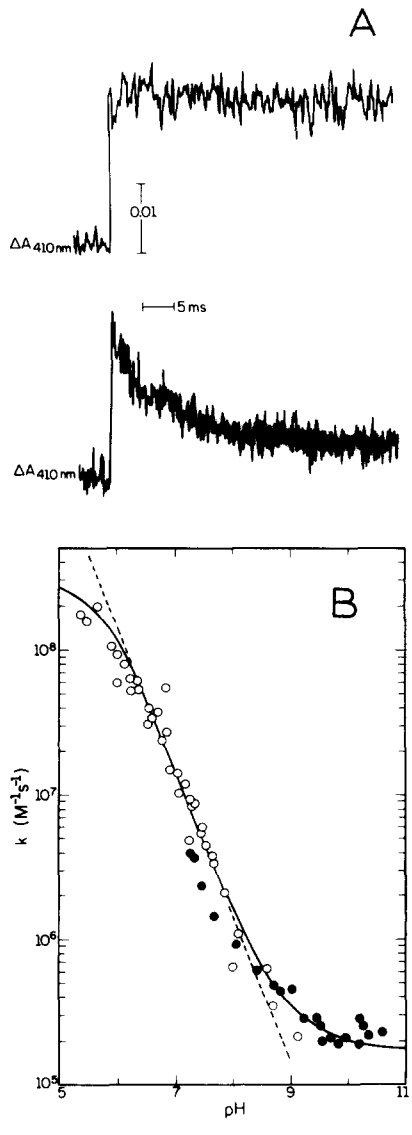


Fig. 6. (A) Direct measure of Q_A^- decay at 410 mM. 2 μM reaction centers, 0.1% Triton X-100, 2 mM *o*-phenanthroline, 100 mM NaCl (pH 7.4). Top: no further additions; bottom: 60 μM ferricyanide and 4 mM ferrocyanide added; $E_h = +290$ mV. (B) Dependence on pH of the second-order rate constant for the oxidation of Q_A^- by ferricyanide. Conditions as for (A) with: (○) 4 mM ferrocyanide and [ferricyanide]$\times \gamma_0 = 6.2$ μM; (●) 10 mM ferrocyanide and [ferricyanide]$\times \gamma_0 = 52$ μM. The rate constants and γ_0 were calculated as in Fig. 3. The solid curve is drawn, as described in the text, according to:

$$k_0^{obs} = k_0' \frac{10^{pK-pH}}{1+10^{pK-pH}} + k_0 \frac{1}{1+10^{pK-pH}}$$

with $k_0' = 2 \cdot 10^8$ $M^{-1} \cdot s^{-1}$, $k_0 = 2 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ and pK = 5.8. The dashed line indicates a slope of 1.

of about $2 \cdot 10^4$ $M^{-1} \cdot s^{-1}$ at pH 6 (Maróti, P., unpublished observations). Thus, in both species, Q_B^- is much less readily oxidized by ferricyanide than is Q_A^-.

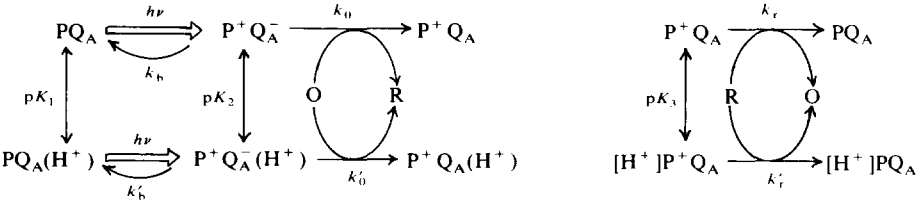
Discussion

In the absence of donors, the decay of the $P^+Q_A^-$ state has generally been interpreted to be a direct intramolecular charge recombination. However, in *Rps. viridis* reaction centers at low pH, in the presence of ferricyanide, Q_A^- is rapidly re-oxidized by ferricyanide before the recombination of P^+ and Q_A^- can occur. The remaining P^+ is then reduced by ferrocyanide. Both processes are shown here to be second-order.

Competition between the pH-sensitive oxidation of Q_A^- by ferricyanide (Fig. 6B) and the much less pH-sensitive recombination reaction [7] gives rise to a pH dependence of the relative amplitudes of the fast (recombination) and slow (donation by ferrocyanide) re-reduction of P^+ (Figs. 1 and 2). The apparent pK of 7.6 for this process (Fig. 2) is very similar to the pK of 7.8 for Q_A^-, evident from the pH dependence of the redox midpoint potential of Q_A [10]. This might be taken to suggest that the rapid oxidation of Q_A^- by ferricyanide is governed by a redox-linked protonation of the quinone complex after the flash. However, we found no difference in the pH dependence of the kinetics of Q_A^- oxidation by ferricyanide whether *o*-phenanthroline was present or not, whereas addition of *o*-phenanthroline to chromatophores is reported to cause a large upward shift in the redox pK of the Q_A/Q_A^- couple, from pH 7.8 to 10.1 [10]. Preliminary measurements on isolated reaction centers show similar behavior for the redox pK (data not shown).

Quantitatively, the protonation-dependent oxidation of Q_A^- by ferricyanide and reduction of P^+ by ferrocyanide account quite well for the observed pH dependence of the amplitudes of the P^+ reduction phases shown in Fig. 2. The standard redox potential span between Q_A/Q_A^- and ferri/ferrocyanide is sufficiently large (greater than 400 mV) that this pathway of Q_A^- oxidation is effectively irreversible. The remaining P^+ is only slowly reducible by ferrocyanide. In the presence of 300 μM ferricyanide, as in Fig. 2, the rate of Q_A^- oxidation by ferricyanide, at pH 9.5, is $0.6 \cdot 10^2 \cdot s^{-1}$ (Fig. 6B). This is 10-times slower than the recombination rate at this pH [7]. Accordingly, at high pH, the titration of Fig. 2 approaches a value of 90% fast rather than 100% fast. At low pH, the titration approaches 10% fast rather than 0% fast. However, this is an artifact of the procedure used to quantitate the two phases in which the amplitude of the fast phase is taken as that amount of P^+ that has decayed by the end of the trace, 13 ms after the flash. Even if all the decay is slow, under the conditions of Fig. 2, roughly 10% reduction of P^+ by ferrocyanide would occur within this 13 ms period. For intermediate pH values, the amplitude of the fast phase of P^+ reduction is determined by competition between the rapid charge recombination and the pH-dependent oxidation of Q_A^- by ferricyanide. The curve in Fig. 2 is drawn according to these limits of 10–90% fast. It does not represent a true titration and the midpoint does not signify the pK of a functional group.

Our results can be summarized by the following schemes:



where O and R refer to ferri- and ferrocyanide, respectively, and (H^+) and $[H^+]$ are different protonable groups. The corrected second-order rate constants are $k_0 = 2 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ and $k'_0 = 2 \cdot 10^8$ $M^{-1} \cdot s^{-1}$ (Fig. 6B); $k_r = 5 \cdot 10^4$ $M^{-1} \cdot s^{-1}$ and $k'_r = 8 \cdot 10^5$ $M^{-1} \cdot s^{-1}$ (Fig. 3B). The recombination rate, k_b, increases slightly with pH ($(0.3–0.6) \cdot 10^3$ s^{-1}), with an inflection at pH 8.1 ± 0.2 [7]. The rapid oxidation of Q_A^- is governed by the population of $P^+Q_A^-(H^+)$ which is determined by a protonation equilibrium with p$K = 5.8$ (see

Fig. 6B). This pK would be equivalent to pK_2 if the proton equilibration was sufficiently fast compared to oxidation of Q_A^- by ferricyanide, but otherwise equal to pK_1. At all pH values, the observed oxidation of Q_A^- by ferricyanide was monophasic, showing that the post-flash proton equilibration is faster than the oxidation rate; thus, p$K_2 = 5.8$. The rate of P^+ reduction by ferrocyanide is modulated by p$K_3 = 7.4$ (see Fig. 3B).

The pH dependence of Q_A^- oxidation by ferricyanide is evidently determined by a protonation equilibrium between a protonated state that reacts readily with ferricyanide and an unprotonated state that is much less reactive. In view of the large negative charge density of ferricyanide, it is not surprising that protonation of the co-reactant, the reaction center, enhances the process. An effect of ionic strength might also be expected and, indeed, the measured rate decreased with increasing ionic strength (data not shown). However, this trend reversed if the substantial correction for the activity coefficient of ferricyanide was applied. There is disagreement in the literature as to whether the ionic strength dependence of small molecule-macromolecule interactions reflects an effective charge at the reactive site or simply the net charge of the whole macromolecule [12–16]. Since the net charge for the *Rps. viridis* reaction center is not known, we are unable to distinguish between these two alternatives. Analysis of the ionic strength dependence by the method of Wherland and Grey [17], which assumes a homogeneous charge distribution, yielded a charge of +4.2 for the reaction center at pH 6.3.

The reduction of P^+ by ferrocyanide in *Rps. viridis* is not especially rapid, having a maximum second-order rate constant (k_r') of 10^6 $M^{-1} \cdot s^{-1}$ below pH 6 (Fig. 3B). This process would appear to be pH-independent below pH 6, but becomes slower at higher pH, with an apparent pK of 7.4 (Fig. 3B). Thus, a protonated form of the reaction center is evidently more reactive. The rate also decreases with increasing ionic strength (data not shown). In reaction centers from *Rhodospirillum rubrum*, the ionic strength dependence of the reduction of P^+ by positively charged, soluble *c*-type cytochromes has been taken to show that this reaction site bears a significant negative charge [18]. In *Rps. viridis* reaction centers, however, analysis of the ionic strength dependence of the ferrocyanide reduction of P^+ by the method of Wherland and Grey [17] indicated a small, positive charge (+3.5 at pH 6.3), similar to that found for the ferricyanide reaction.

The rate of Q_B^- oxidation by ferricyanide was considerably slower than that of Q_A^-, indicating a lower reactivity or accessibility for ferricyanide at that site. This is contrary to a simple expectation from the X-ray structure of this protein, which indicates Q_A to be at least as, or more, deeply buried than Q_B (Ref. 2, and Michel, H., personal communication). However, the X-ray structure, at the present resolution, does not reveal the charge density at these sites or the net charge of the reaction center, both of which may be important in modulating reactivity.

Since *o*-phenanthroline and Q_B bind competitively, probably at the same site (Ref. 19, and Michel, H., personal communication), the lack of effect of *o*-phenanthroline on the oxidation rate of Q_A^- by ferricyanide strongly suggests that ferricyanide does not act via the Q_B site. Moreover, Q_B^- oxidation by ferricyanide is much slower than oxidation of Q_A^-. The relative reactivities of Q_A^- (k_0^A) and Q_B^- (k_0^B) to ferricyanide, in both *Rps. viridis* and *Rps. sphaeroides*, are very similar to the known equilibrium constant (K_2) for electron-sharing between Q_A^- Q_B and Q_A Q_B^- [7,19,20,22]. Ferricyanide may, therefore, be directly reactive only with Q_A^-; Q_B^- would then be oxidized by ferricyanide indirectly, via Q_A. In this case, the ratio of oxidation rates is given by:

$$k_0^A/k_0^B = 1 + K_2$$

A similar relationship has been used to describe the kinetics of recombination for the states $P^+Q_A^-$ and $P^+Q_B^-$ and has provided values of $K_2 = 100$ for *Rps. viridis* [7] and 15 for *Rps. sphaeroides* [20,22]. From the observed rates of Q_A^- and Q_B^- oxidation by ferricyanide, the ratio k_0^A/k_0^B, at pH 6, is approx. 75 for *Rps. viridis* and 20 for *Rps. sphaeroides*, in reasonable agreement with the expectations of this model.

These studies on *Rps. viridis*, show that ferricyanide can be a very reactive, possibly diffusion-limited, oxidant of Q_A^- when the reaction center is appropriately protonated.

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