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The acceptor quinone complex of *Rhodopseudomonas viridis* reaction centers

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The acceptor complex of isolated reaction centers from *Rhodopseudomonas viridis* contains both menaquinone and ubiquinone. In a series of flashes the ubiquinone was observed to undergo binary oscillations in the formation and disappearance of a semiquinone, indicative of secondary acceptor (Q_B) activity. The oscillating signal, Q_B^-, was typical of a ubisemiquinone anion with a peak at 450 nm ($\Delta\epsilon = 6$ mM$^{-1}\cdot$cm^{-1}) and a shoulder at 430 nm. Weak electrochromic bandshifts in the infrared were also evident. The spectrum of the reduced primary acceptor (Q_A^-) exhibited a major peak at 412 nm ($\Delta\epsilon = 10$ mM$^{-1}\cdot$cm^{-1}) consistent with the assignment of menaquinone as Q_A. The Q_A^- spectrum also had minor peaks at 385 and 455 nm in the blue region. The same spectrum was recorded after quantitative removal of the secondary acceptor, when only menaquinone was present in the reaction centers. Spectral features in the near-infrared due to Q_A^- were attributed to electrochromic effects on bacteriochlorophyll (BChl) *b* and bacteriopheophytin (BPh) *b* pigments resulting in a distinctive split peak at 810 and 830 nm ($\Delta\epsilon = 8$ mM$^{-1}\cdot$cm^{-1}). The menaquinone was identified as 2-methyl-3-nonylisoprenyl-1,4-naphthoquinone (menaquinone-9). The native Q_A activity was uniquely provided by this menaquinone and ubiquinone was not involved. Q_B activity, on the other hand, displayed at least a 40-fold preference for ubiquinone (Q-10) as compared to menaquinone. Thus, both quinone-binding sites display remarkable specificity for their respective quinones. In the absence of donors to P^+, charge recombination of the $P^+Q_A^-$ and $P^+Q_B^-$ pairs had half-times of 1.1 ± 0.2 and 110 ± 20 ms, respectively, at pH 9.0, indicating an electron-transfer equilibrium constant (K_2^{app}) of at least 100 for $Q_A^-Q_B \rightleftharpoons Q_AQ_B^-$. Also observed was a slow recombination of the cytochrome *c*-558$^+$ Q_A^- pair, with $t_{1/2} = 2 \pm 0.5$ s at pH 6.

Introduction

The purple non-sulfur photosynthetic bacterium, *Rhodopseudomonas viridis*, utilizes bacteriochlorophyll (BChl) *b* instead of BChl *a*, but in most other respects is similar to other BChl-*a*-containing purple, photosynthetic bacteria. The reaction center consists of four subunits [1]. The largest of these (40 kDa) probably contains four bound hemes: two low-potential cytochromes (cyt *c*-553) and two high-potential cytochromes (cyt *c*-558) [1,2]. The other three subunits seem to correlate with the L, M and H subunits of the better studied *Rps. sphaeroides* reaction center [1,3]. The physicochemical properties of the primary donor (P) and acceptor components are also common to

Abbrevations: BChl, bacteriochlorophyll; BPh, bacteriopheophytin; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylene diamine; MK, menaquinone; UQ, ubiquinone; PMS, *N*-methylphenazonium methosulfate; LDAO, lauryldimethylamine *N*-oxide.

other purple bacteria studied so far.

The presence of ubiquinone and a menaquinone-like chemical in subchromatophore particles from *Rps. viridis* has been reported [4]. Subsequently, Pucheu et al. [5] found only menaquinone associated with isolated reaction centers, while ubiquinone dominated in the chromatophore membrane. This implied that menaquinone was involved in primary activity and suggested a pool and possible secondary acceptory activity for ubiquinone. Purification of the reaction centers tends to remove the secondary quinone [3,6] and Trosper [7] has found variable levels of menaquinone and ubiquinone in *Rps. viridis* reaction centers isolated by different methods. The first stable acceptors have been partially characterized, by electron paramagnetic resonance, as quinones coupled to an iron [8,9] as observed in other photosynthetic bacteria and in Photosystem II of plants [3,10]. In this paper we clarify the chemical identity of the menaquinone and the roles of menaquinone and ubiquinone as electron acceptors in reaction centers of *Rps. viridis*.

The kinetics of the $P^+Q_A^-$ recombination in *Rps. viridis* has been largely studied in chromatophores by the indirect technique of delayed fluorescence. Using three flashes in rapid succession, to oxidize the two cyt *c*-558 and set-up the state $P^+Q_A^-$, Fleischman and coworkers 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 [11]. Carithers and Parson [12] confirmed the main decay rate of $10^3\ s^{-1}$ for $P^+Q_A^-$ in chromatophores under controlled conditions. We report here the decay kinetics of $P^+Q_A^-$ in isolated reation centers and also the recombination rate for the $P^+Q_B^-$ pair.

Materials and Methods

Cells were grown in 13 l batches in Hutner's medium, under 0.3 $mE \cdot m^{-2} \cdot s^{-1}$ illumination (tungsten filament bulbs), and harvested after 3–4 days growth. Reaction centers and chromatophores were prepared as described by Prince et al. [8]. The ratio of A_{280}/A_{830} was routinely 2.1–2.4 for the reaction centers. These reaction centers were observed to have four subunits by sodium dodecyl sulfate polyacylamide gel electrophoresis. All kinetic experiments were performed under anaerobic conditions with controlled pH and redox potential. The redox potential was adjusted by the addition of ferricyanide, ferrocyanide or ascorbate; other redox mediators used were diaminodurene and *N,N,N',N'*-tetramethyl-*p*-phenylene diamine (TMPD). The actinic light was a xenon flash lamp (EG and G, FX-200), filtered with a Kodak Wratten 70 gelatin filter, and was more than 95% saturating. The photomultiplier (Hamamatsu, R928) was protected by either a Corning 4-76 or a Wratten 29a filter. The monochromator (Bausch and Lomb, 0.5 m) was calibrated using a mercury-arc source, a He-Ne laser and various interference filters. Reaction center concentrations were determined using $\Delta\epsilon_{960\ red\text{-}ox} = 123\ mM^{-1} \cdot cm^{-1}$ [2].

Menaquinone (MK) and ubiquinone (UQ) were isolated from whole cells or reaction centers by extraction with light petroleum ether/methanol (3 : 2, v/v) and further purified by silica gel thin layer chromatography (TLC) [13,14]. The quinones were characterized by their ultraviolet spectra when oxidized and quantified by reduced-minus-oxidized absorption difference [14,15]. To determine the nature of the isoprenoid chain for the purified menaquinone isolated from *Rps. viridis*, mass spectroscopy was utilized to determine the molecular weight and $AgNO_3$ silica gel TLC, with 5% methanol in benzene as solvent, to determine the degree of saturation [14]. Standards used were MK-8 isolated from *E. coli* [13,14], bacterial Q-10 (Calbiochem.), Vitamin K1 (Eastman Kodak) and synthetic MK-8 (a gift from Hoffman-LaRoche).

Secondary acceptor quinone was extracted from the reaction centers by the method of Okamura et al. [16]. Reaction centers treated as such are referred to as Q_B-less. When appropriate, Q-10 or MK-8 was added to reaction centers from a stock solution (10 mM) in 30% Triton X-100 prepared by sonication.

Results

Identification of the primary acceptor

With an exogenous donor, such as diaminodurene or TMPD, to rereduce the oxidized cyt *c*-558, a stable absorbance signal remained after flash activation of Q_B-less *Rps. viridis* reac-

tion centers (Fig. 1a). This spectrum is attributable to the reduction of the primary acceptor, Q_A^-. The blue portion of the spectrum was dominated by a peak at 412 nm with $\Delta\epsilon = 10\ mM^{-1} \cdot cm^{-1}$. The peak wavelength did not shift with pH between pH 6–10. In vitro, unprotonated menasemiquinone has been reported to have a single broad peak at 395 nm with $\Delta\epsilon = 11\ mM^{-1} \cdot cm^{-1}$ and to show a 25 nm blue shift of the absorbance peak when protonated [17]. The spectrum of the reduced primary acceptor of *Chromatium vinosum*, believed to be MK-7, was reported to have a major peak at 400 nm with $\Delta\epsilon = 10.8\ mM^{-1} \cdot cm^{-1}$ [18]. In *Rps. viridis* reaction centers, additional minor peaks in the blue region were observed at 385 and 455 nm (Fig. 1a). Similar peaks were previously observed for *C. vinosum* [18]. The presence of a peak at 455 nm raised the possibility of partial involvement of UQ in primary activity, but analysis of the Q_B-less reaction centers established that only menaquinone was present in these preparations (ubiquinone was below the detection level of 0.1 Q per reaction center). The same spectrum was observed in unextracted, Q_B-containing reaction centers, in the presence of *o*-phenanthroline to inhibit electron transfer from Q_A^- to Q_B. Thus, the 455 nm peak is a true attribute of menasemiquinone as Q_A^- (or an accompanying electrochromic effect) and the primary acceptor activity of *Rps. viridis* is evidently provided by MK alone even when UQ is present.

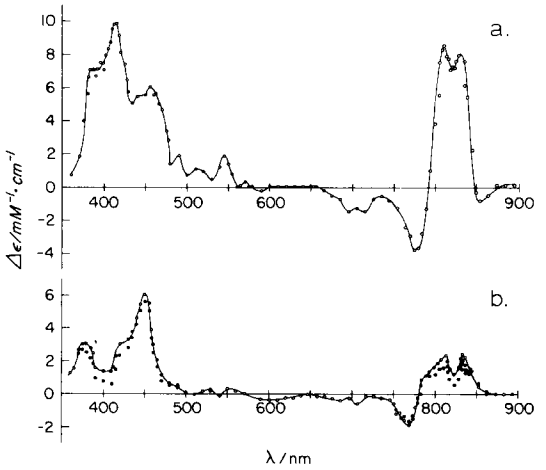


Fig. 1. Difference spectra for photoreduction of primary acceptor, Q_A, and secondary acceptor, Q_B. (a) Q_A: 2 μM Q_B-less reaction centers/2 mM diaminodurene/10 mM Tris-HCl (pH 8.6) $E_h = +150$ mV, 0.06% Triton X-100; (b) Q_B: (○) same as (a) but with 20 μM Q-10, $E_h = +180$ mV and pH = 8.0; (●) corrected for 7% Q_A^- present due to that fraction of reaction centers in which Q_B activity could not be reconstituted. Both are corrected for 12.5% double hits, determined by comparison of single-flash cytochrome *c*-558 oxidation in the presence and absence of 4 mM *o*-phenanthroline.

Reduction of the primary acceptor also induced absorbance changes in the near-infrared, indicative of electrochromic effects on nearby chromophores (Fig. 1a). The near infrared spectrum for Q_A^- in *Rps. viridis* appears to result from at least two effects: a red shift of a bacteriopheophytin (BPh) *b* centered at 790–795 ($\Delta\epsilon = 8\ mM^{-1} \cdot cm^{-1}$ at 810 nm) and an apparent band narrowing of a BChl *b* absorption peak at 830 nm.

In the presence of diaminodurene (1.5 mM) to rereduce the cyt *c*-558, the flash induced menasemiquinone in Q_B-less reaction centers of *Rps. viridis* had a half-time of decay of 80 s at pH 9. When *o*-phenanthroline (4 mM) was added this half-time decreased to 35 s. Under similar conditions the decay half-times of Q_A^- in Q_B-less reaction centers from *Rps. sphaeroides* R-26 were 130 and 60 s in the absence and presence of *o*-phenanthroline, respectively.

The menaquinone from whole cells of *Rps. viridis* was isolated and purified as outlined in Materials and Methods. The resulting oxidized MK had the distinctive absorption spectrum of a 2-methyl-3-polyisoprenyl-1,4-naphthoquinone [14]. The MK from *Rps. viridis* was run on an analytical silica gel TLC plate incorporated with $AgNO_3$. This technique allows separation of similar compounds on the basis of the degree of unsaturation [14], in this case the number of isoprenyl units in the side chain. Comparison with standards (Q-10, MK-8 and Vitamin K1) indicated that the menaquinone from *Rps. viridis* was MK-9, as suggested by an earlier study [9]. This assignment was confirmed by mass spectroscopy which gave a high mass peak of $m/e = 784$, corresponding to the molecular weight of MK-9. Synthetic MK-8, as a standard, had a high mass peak of $m/e = 716$, equivalent to one less isoprenoid unit than the MK from *Rps. viridis*.

Identification of the secondary acceptor

Secondary acceptor activity can be readily restored in isolated *Rps. viridis* reaction centers by the addition of ubiquinone (Q-10) (Fig. 2a). The binary oscillations of Q_B^- were inhibited by *o*-phenanthroline (Fig. 2b). The spectrum for the formation of ubisemiquinone as Q_B^- was obtained from the oscillating, stable absorbance changes elicited by a series of flashes in the presence of exogenous donors (Fig. 1b). The blue region of the spectrum was typical of a bound, unprotonated ubisemiquinone, as previously reported for Q_B^- of *Rps. sphaeroides* [20,21], with a peak at 450 nm ($\Delta\epsilon = 6\ mM^{-1} \cdot cm^{-1}$) and a prominent shoulder at 435 nm (Fig. 1b). In addition, some features were observable in the near-infrared, the most prominent being a bandshift centered at 780–785 nm ($\Delta\epsilon = 2\ mM^{-1} \cdot cm^{-1}$), attributable to a BPh *b*. During preparation of the reaction centers most of the secondary acceptor was usually removed but some preparations retained a significant fraction of the native secondary acceptor. The blue portion of the spectrum for native Q_B^- was identical in shape to that shown in Fig. 1b, indicating that the native secondary acceptor was indeed a ubiquinone. The stable ubisemiquinone of *Rps. viridis*, Q_B^-, in the presence of diaminodurene (1.5 mM) to rereduce to cyt *c*-558, decayed with a half-time of 65 s. Under similar conditions the halftime for Q_B^- decay in *Rps. sphaeroides* R-26 reaction centers was 140 s, somewhat slower than that found here for *Rps. viridis*. This is, however, considerably faster than that reported earlier for *Rps. sphaeroides* [21,22], probably because of more effective redox mediation by the high concentration of diaminodurene used here.

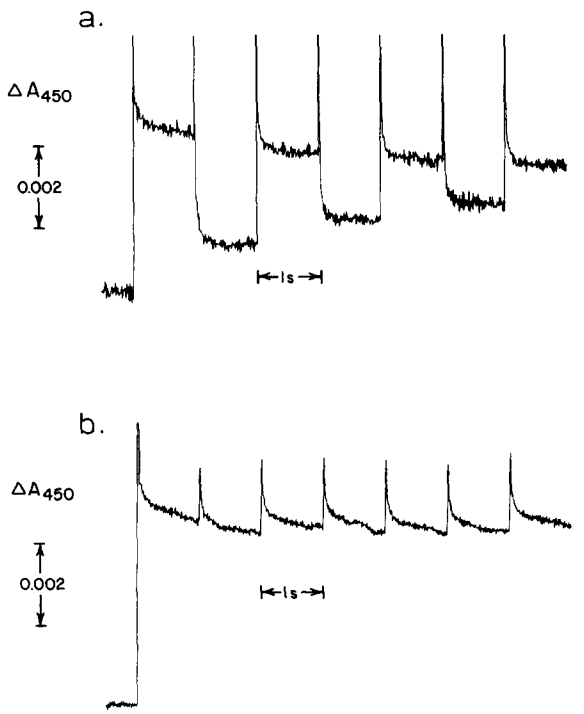


Fig. 2. Absorption changes at 450 nm in a series of flashes. 0.65 μM reaction centers/20 μM Q-10/200 μM TMPD/200 μM diaminodurene; at these concentrations of TMPD and diaminodurene, some rereduction of cyt *c*-558 is still visible on this timescale ($t_{1/2} \approx 100$ ms), giving rise to a small absorbance decrease on top of the stable semiquinone signals. These measurements were made at pH 10 where the $Q_A \rightarrow Q_B$ electron transfer is sufficiently slow to eliminate double turnovers during a flash. (a) No further additions; (b) plus 2 mM *o*-phenanthroline.

There is a considerable specificity for ubiquinone as the secondary acceptor, compared to menaquinone. Secondary acceptor activity can be determined from the extent of cytochrome oxidation on a second flash [23]. From a quinone concentration dependence of this, the apparent binding constant (K_q) of Q-10 to the Q_B site after a flash was found to be 1–2 μM, whereas K_q for MK-8 was approx. 80 μM (data not shown).

The secondary acceptor activity in *Rps. viridis* was susceptible to Photosystem II herbicides (Table I) with the same selectivity exhibited by *Rps. sphaeroides* [24].

Charge recombination kinetics of $P^+Q_A^-$ and $P^+Q_B^-$

The decay kinetics of P^+ were obtained at 450 nm. The rate of recombination of P^+ and Q_A^- was measured in a sample in which the cyt *c*-558 was largely oxidized by ambient O_2 without the addition of ferricyanide. We have observed that ferricyanide can rapidly oxidize Q_A^- in isolated reaction centers from *Rps. viridis* [25] and this oxidant must be excluded from measurements of the $P^+Q_A^-$ recombination kinetics. Secondary electron transfer to Q_B was eliminated either by the addition of *o*-phenanthroline (2–4 mM) or by quantitative removal of secondary quinone and usually by both.

TABLE I

COMPARISON OF HERBICIDE ACTIVITY IN *RPS. SPHAEROIDES* AND *RPS. VIRIDIS*

I_{50} measured from extent of cytochrome *c* photooxidation after two flashes. Conditions for *Rps. viridis*: 2 μM reaction centers/20 μM Q-10/0.1% Triton X-100/10 mM Tris (pH 8). Data for *Rps. sphaeroides* from Ref. 24; 1 μM reaction centers /20 μM Q-10/0.06% Triton X-100/100 mM NaCl/10 mM Tris (pH 8). Herbicide names are defined as in Weed Science Society of America, Herbicide Handbook, 4th Ed., 1979.

Inhibitor	I_{50}	
	Rps. sphaeroides (μM)	*Rps. viridis* (μM)
Ametryn	20	15
Atrazine	120	40
Terbutryn	3	2
Bromoxynil	30	20
o-Phenanthroline	215	80
Diuron	no effect	no effect
Bromacil	no effect	no effect

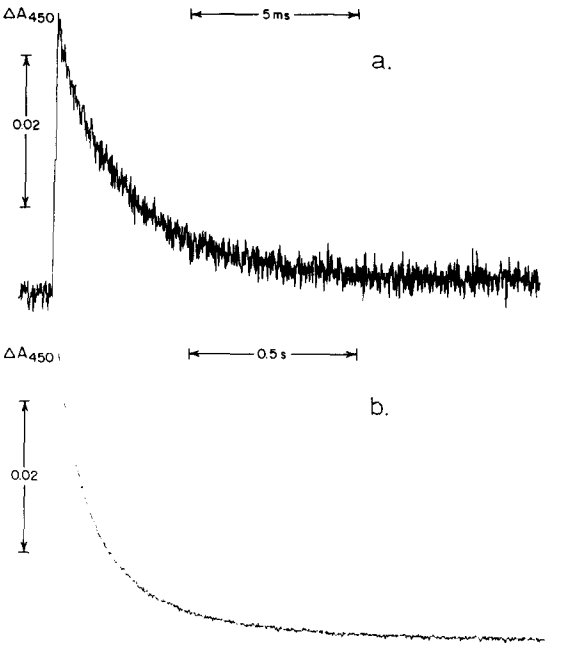


Fig. 3. Charge recombination kinetics measured at 450 nm. (a) $P^+Q_A^- \rightarrow PQ_A$; 0.1% Triton X-100/4 mM *o*-phenanthroline/10 mM Tris (pH 9)/1.16 μM reaction centers (less than 0.1 μM ferricyanide after repeated dilution and ultrafiltration). (b) $P^+Q_B^- \rightarrow PQ_B$; same as (a) but with 20 μM Q-10 and no *o*-phenanthroline.

At pH 9.0, and above, the recombination halftime was 1.1 ± 0.2 ms (Fig. 3a). The half-time of the backreaction increased steadily as the pH was lowered, levelling off to 2.0 ms at pH 6.0, and below, with an inflection at about pH 8. Identical pH-dependences were observed both in the presence and absence of *o*-phenanthroline. This behavior was confirmed in samples which were further oxidized by ferricyanide, followed by removal of the ferricyanide by repeated dilution and ultrafiltration. Similar results were also obtained using the method of Fleischman [11], giving three flashes in the presence of *o*-phenanthroline to observe the $P^+Q_A^-$ recombination. In the absence of *o*-phenanthroline, the addition of Q-10 induced a much slower decay kinetic for P^+. The half-time for this decay, attributed to the recombination of $P^+Q_B^-$, was 110 ms ± 20 ms at pH 9.0 (Fig. 3b).

Charge recombination kinetics of cytochrome c-558$^+Q_A^-$

To determine the rate of decay of Q_A^- when the primary donor was reduced we used the double flash method of Parson [23], at a redox potential of +250 mV where the bound high-potential cytochrome *c*-558 is fully reduced. The amount of cytochrome oxidized by the second flash is a measure of the extent Q_A^- reoxidation. With Q_B-less reaction centers the amount of cytochrome oxidized by the second flash was equal to the amount of cytochrome that rereduced in the dark following the first flash, (Fig. 4a). Thus Q_A^- and cyt *c*-558$^+$ decay with the same kinetics. The half-time at pH 6.1 was 2 ± 0.5 s (Fig. 4b) and did not change when the reaction center concentration was varied 40-fold (0.1–4.0 μM). A mild pH dependence, however, was observed and the recombination at pH 8 was twice as fast. The concentration insensitive, parallel kinetics imply that the cyt *c*-558$^+$ Q_A^- pair slowly recombine by an intramolecular process. A concentration independent decay of reduced primary acceptor has been reported in *C. vinosum* subchromatophore particles, with a half-time of 200 ms [26]. Although the time dependence of cyt c^+ decay was not reported, a similar, albeit faster, charge recombination might be suspected in this species as well.

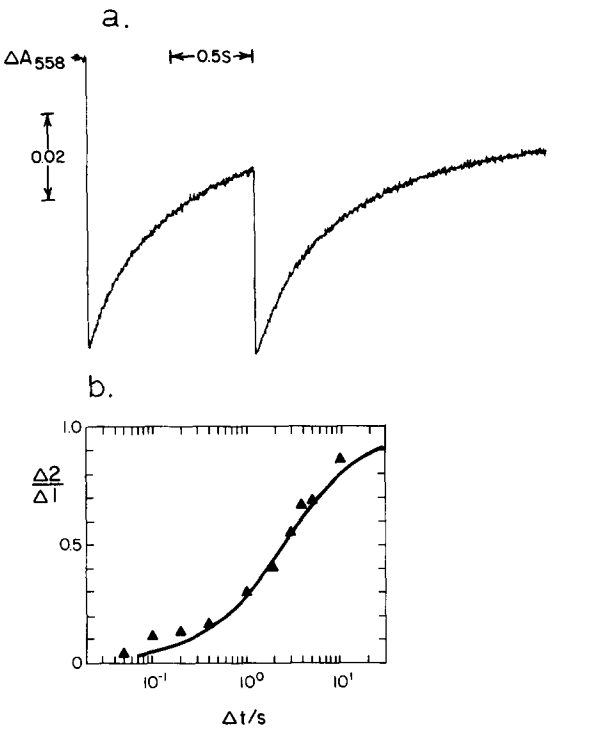


Fig. 4. Charge recombination of cyt c-558$^+$ Q_A^-. (a) Kinetic trace of two consecutive flashes at 558 nm; 4 μM reaction centers/0.1% LDAO/2 mM o-phenanthroline/100 mM NaCl/1 mM Tris (pH 8)/20 μM ascorbate; $E_h = +200$ mV. (b) Plot of ratio of the second flash cyt c-558 oxidation to the first flash oxidation vs. dark time between flashes. 1.3 μM reaction centers/400 μM o-phenanthroline/1 mM potassium ferrocyanide/100 mM NaCl/0.03% LDAO (pH 6.1) $E_h = +250$ mV.

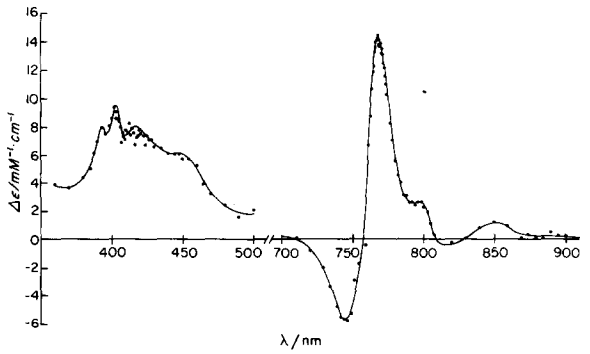


Fig. 5. Difference spectrum for photoreduction of menaquinone as Q_A in *Rps. sphaeroides* R-26 reaction centers; 0.1% Triton X-100/2 mM diaminodurene/10 mM Tris (pH 8.5)/10 μM MK-8/4 mM o-phenanthroline/1.5 μM Q-less reaction centers.

Menaquinone as Q_A in Rps. sphaeroides

The naturally occurring quinone in *Rps. sphaeroides* reaction centers is Q-10. To make a more apt comparison between the species *Rps. sphaeroides* R-26 and the menaquinone-containing *Rps. viridis*, we removed 98% of the primary quinone from R-26 reaction centers by the method of Okamura et al. [16] and substituted MK-8 as the primary quinone. The spectrum of MK-8 as Q_A^- (Fig. 5) was similar, in the blue region, to that of Q_A^- in *Rps. viridis*, with a major peak at 405 and a significant shoulder at 450 nm. In the near infrared region a bandshift of a BPh *a* can be clearly observed as well as other less prominent bandshifts. The half-time for the recombination of P^+ Q_A^-, at pH 8, was 42 ± 2 ms for MK-8 as Q_A in *Rps. sphaeroides* reaction centers, compared to 75 ms for native Q-10 as Q_A. With MK-8 as Q_A and Q-10 as Q_B, the recombination halftime for $P^+Q_B^-$ was 1.16 ± 0.02 s. Under equivalent conditions of detergent (0.3% Triton X-100) and ubiquinone concentration (20 μM) the half-time for R-26 reaction centers, with Q-10 functioning as both Q_A and Q_B, was 450 ms.

Discussion

The first part of this work clarifies the roles of menaquinone and ubiquinone in the reaction center of *Rps. viridis*. The roles are not shared by the two different quinone types but are quite distinct. When reaction centers are isolated from membranes containing significant amounts of both quinones, MK is uniquely active as the primary acceptor, while UQ acts as the secondary quinone. This assignment of activity has been suspected for both *Rps. viridis* and *C. vinosum* but the possibility of shared activity had not previously been ruled out. Since ubiquinone is present in excess over menaquinone it is evident that the binding site for Q_A (A-site) displays considerable specificity for the native quinone. The specificity of the B-site of *Rps. viridis* for UQ, compared to MK, was shown by direct comparison. The B-site in *Rps. viridis* is evidently similar to that in *Rps. sphaeroides* since the sensitivity to chemically distinct inhibitors displays the same trend of effectiveness. The slightly greater effectiveness of the herbicides in *Rps. viridis* is accountable by the different conditions of the

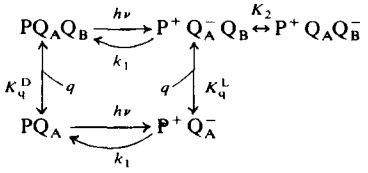
experiment, lower UQ/RC and higher detergent levels, rather than any inherent species difference.

The structure in the blue region of the Q_A^- spectrum (Fig. 1a) probably results from several minor bandshifts and it is not clear where the true peak of the menasemiquinone lies. However the shape of the absorbance band, especially the short wavelength edge, suggests a red shift of about 10–15 nm compared to the in vitro menasemiquinone [17,18]. Red shifts are seen in all other spectra of bound semiquinones in reaction centers observed so far [18,21,27–29], although this is sometimes only apparent in the ultraviolet peaks at 280–320 nm. The apparent lack of a redshift of the blue (395 nm) peak of Q_A^- in *C. vinosum* [18] and *Chloroflexus aurantiacus* [30] may be due to the complexity of the spectrum in this region with overlapping bandshifts and, perhaps, contributions from exogenous donors. We have found *N*-methylphenazonium methosulfate (PMS) (used in Refs. 18 and 30) to be particularly troublesome in this regard. We originally reported the peak for Q_A^- in *Rps. viridis* to lie at 390 mm [31], but subsequently discovered this to arise from a distortion of the peak by residual, oxidized PMS radical absorption contributions in this region.

A prominent peak at 445–455 nm has been observed in the Q_A^- spectrum of three different species where menaquinone was reported to act as Q_A^-: *Rps. viridis* (this work), *C. vinosum* [18] and *Cf. aurantiacus* [30], a species which contains ony menaquinone [32]. The chemical identity of the pigment responsible for this spectral contribution is unknown, although a carotenoid might perhaps be an obvious candidate. However, when *Rps. viridis* reaction centers were washed with 4% LDAO, a procedure reported to remove the carotenoid [1], the peak at 455 nm in the Q_A^- spectrum remained (not shown). Furthermore, a similar shoulder, at about 450 nm, in the menasemiquinone spectrum was seen when MK-8 was supplied as the primary quinone in reaction centers from *Rps. sphaeroides* R-26, a carotenoidless mutant. These two results seem to rule out the involvement of a carotenoid in the 450–455 nm peak and supports instead the notion that it is intrinsic to the menasemiquinone in vivo. In vitro the menasemiquinone exhibits an unusually extended red edge to the main absorption peak [17]. Enhancement of this region could be responsible for the 450 nm shoulder seen in vivo.

The red shift of the infrared absorbance band of a BPh *b*, caused by Q_A^-, is very similar to the effect of Q_A^- on a BPh *a* in *Rps. sphaeroides*, indicating similarities between the two species in the geometric arrangement of a BPh relative to the primary quinone. The midpoint of the bandshift identifies this as the longer-wavelength-absorbing of the two BPh, which has been established as the intermediate electron acceptor, I, the direct reductant of Q_A [33]. The electrochromic influence of Q_B^- on the near infrared spectrum of *Rps. viridis* reaction centers shows a dominant effect on the shorter wavelength BPh *b*. The perturbation by Q_A^- of a BChl *b* at 830 nm gives rise to an easily discernable peak in *Rps. viridis*, whereas in *Rps. sphaeroides* an equivalent bandshift of BChl *a* appears as a weak shoulder [34] or is not apparent at all [35].

Our observations on the $P^+Q_A^-$ recombination rate supports earlier data from chromatophore studies, although the rate in isolated reaction centers appears to be slightly slower. This effect of isolation is also observed in *Rps. sphaeroides* reaction centers [24]. The slower P^+ decay kinetic in the presence of excess Q-10 was interpreted to be due to the $P^+Q_B^-$ recombination, which has been proposed to occur by an indirect pathway via the equilibrium with the $P^+Q_A^-$ state [36–38]. When the binding equilibrium of the quinone at the Q_B site is considered, the overall scheme becomes:



where q is free ubiquinone. The dominance of this indirect recombination pathway in *Rps. sphaeroides* reaction centers has been thoroughly established by Kleinfeld et al. [39]. From this model the relationship between the half-times of charge recombination and the equilibrium constant is [38]:

$$t_{1/2}^{Q_B} = t_{1/2}^{Q_A}\left(1 + K_2\frac{[q]/K_q^L}{1+[q]/K_q^L}\right) = t_{1/2}^{Q_A}[1 + K_2^{app}]$$

At high [Q] (as in Fig. 3b), this tends towards:

$$t_{1/2}^{Q_B} = t_{1/2}^{Q_A}(1 + K_2)$$

From the half-times reported here, $t_{1/2}^{Q_A} = 1.1$ ms and $t_{1/2}^{Q_B} = 110$ ms, we find $K_2^{app} = 100$ at pH 9. This value is nearly an order of magnitude greater than that found for *Rps. sphaeroides* reaction centers $K_2^{app} = 10$–15 in 0.1% Triton X-100 [36,38–40], indicating a greater redox potential difference between menaquinone as Q_A and ubiquinone as Q_B in *Rps. viridis* compared to the two ubiquinone acceptors in *Rps. sphaeroides*. This conclusion is consistent with equilibrium redox titrations of Q_A/Q_A^- and Q_B/Q_B^- in these two species [9,37,41]. The rapid recombination for $P^+Q_A^-$ in *Rps. viridis* ($t_{1/2}^{Q_A} = 1.1$ ms) could not be duplicated in *Rps. sphaeroides* reaction centers simply by inserting MK-8 as Q_A, although the lower redox potential MK-8 did give a faster recombination than the natural Q-10. In addition, the large K_2^{app} (≈ 100) for *Rps. viridis* could not be matched by the mixed quinone system (MK as Q_A, Q-10 as Q_B) in *Rps. sphaeroides*, which yielded a $K_2^{app} \approx 25$, only slightly higher than that for the native quinone configuration under similar conditions. Thus the distinctions between *Rps. viridis* and *Rps. sphaeroides* are not due simply to MK vs. UQ as Q_A, but must include differences in reaction center structure.

The decay kinetics found here for the state $P^+Q_B^-$, ($t_{1/2}^{Q_B} = 110$ ms), in isolated reaction centers of *Rps. viridis*, are faster than what is observed in chromatophores, $t_{1/2} = 400$ ms (Fleischman, D.E., personal communication), for apparently the same process. A similar discrepancy is seen in *Rps. sphaeroides* [24,34,37]. In part this may arise from a lower effective quinone concentration in the reaction center experiments in detergent solutions (1–2 Q per micelle) compared to the quinone concentration in the chromatophore membrane (≈ 25 Q/RC), effectively lowering K_2^{app} in the isolated reaction center. However, an additional factor may be the nature of the hydrophobic environment (membrane vs. micelle) affecting the conformational structure of the reaction center. From the decay half-times we find $K_2^{app} \approx 500$ for chromatophores and $K_2^{app} \approx 100$ for reaction centers. Both these values are an order of magnitude greater than those reported for *Rps. sphaeroides* [24].

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