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Is bicarbonate in Photosystem II the equivalent of the glutamate ligand to the iron atom in bacterial reaction centers?

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Photosystem II of oxygen-evolving organisms exhibits a bicarbonate-reversible formate effect on electron transfer between the primary and secondary acceptor quinones, Q_A and Q_B . This effect is absent in the otherwise similar electron acceptor complex of purple bacteria, e.g., *Rhodobacter sphaeroides*. This distinction has led to the suggestion that the iron atom of the acceptor quinone complex in PS II might lack the fifth and sixth ligands provided in the bacterial reaction center (RC) by a glutamate residue at position 234 of the M-subunit in *Rb. sphaeroides* RCs (M232 in *Rps. viridis*). By site-directed mutagenesis we have altered Glu^{M234} in RCs from *Rb. sphaeroides*, replacing it with valine, glutamine and glycine to form mutants M234EV, M234EQ and M234EG, respectively. These mutants grew competently under phototrophic conditions and were tested for the formate-bicarbonate effect. In chromatophores there were no detectable differences between wild type (Wt) and mutant M234EV with respect to cytochrome *b*-561 reduction following a flash, and no effect of bicarbonate depletion (by incubation with formate). In isolated RCs, several electron transfer activities were essentially unchanged in Wt and M234EV, M234EQ and M234EG mutants, and no formate-bicarbonate effect was observed on: (a) the fast or slow phases of recovery of the oxidized primary donor (P^+) in the absence of exogenous donor, i.e., the recombination of $P^+Q_A^-$ or $P^+Q_B^-$, respectively; (b) the kinetics of electron transfer from Q_A^- to Q_B^- ; or (c) the flash dependent oscillations of semiquinone formation in the presence of donor to P^+ (Q_B turnover). The absence of a formate-bicarbonate effect in these mutants suggests that Glu^{M234} is not responsible for the absence of the formate-bicarbonate effect in Wt bacterial RCs, or at least that other factors must be taken into account. The mutant RCs were also examined for the fast primary electron transfer along the active (A)-branch of the pigment chain, leading to reduction of Q_A . The kinetics were resolved to reveal the reduction of the monomer bacteriochlorophyll ($\tau = 3.5$ ps), followed by reduction of the bacteriopheophytin ($\tau = 0.9$ ps). Both steps were essentially unaltered from the wild type. However, the rate of reduction of Q_A was slowed by a factor of 2 ($\tau = 410 \pm 30$ and 47 ± 30 ps for M234EQ and M234EV, respectively, compared to 220 ps in the wild type). EPR studies of the isolated RCs showed a characteristic $g = 1.82$ signal for the Q_A^- semiquinone coupled to the iron atom, which was indistinguishable from the wild type. It is concluded that Glu^{M234} is not essential to the normal functioning of the acceptor quinone complex in bacterial RCs and that the role of bicarbonate in PS II is distinct from the role of this residue in bacterial RCs.

Introduction

Electron flow through the quinone electron acceptors of Photosystem II (PS II) in oxygen-evolving organisms is inhibited by several monovalent anions, of which formate is one of the most active [1]. Addition of bicarbonate reverses this inhibition producing a large stimulation of electron transfer from Q_A , the primary

quinone, to Q_B , the secondary quinone acceptor of PS II [2]. It is considered that bicarbonate rather than CO_2 is the species required for the stimulation [3]. The antagonistic action of formate and bicarbonate on electron transfer is termed the bicarbonate effect and is considered to be a manifestation of a genuine bicarbonate requirement. However, the effect is difficult to demonstrate directly by depletion in the absence of formate [4]. Bicarbonate depletion results in a small (2–3-fold) decrease in the rate of the first electron transfer from Q_A^- to Q_B , a more marked inhibition of the second electron transfer from Q_A^- to Q_B^- and a

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significant inhibition of the release of O_2H_2 from the reaction center.

The site of the bicarbonate effect has been shown to be the acceptor quinone complex of PS II [4]. However, in purple bacteria, which possess a functionally very similar acceptor quinone complex to that of PS II, no bicarbonate effect could be demonstrated, either in isolated reaction centers (RCs) or in chromatophores of *Rhodobacter sphaeroides* [5]. The X-ray crystallographic structures of RCs from *Rhodospseudomonas viridis* [6] and *Rb. sphaeroides* [7,8] are known, and the acceptor quinone complex is seen to include a non-haem iron coordinated by four histidines and by a bidentate ligand interaction with the carboxylate group of a glutamate residue (M234 in the *Rb. sphaeroides* sequence). Comparison of the amino-acid sequences of RC proteins from PS II indicates that the subunit structure of PS II and purple bacterial RCs are roughly homologous, with D1 and D2 of PS II equivalent to the L and M subunits of bacterial RC. Amino-acid sequence differences in the polypeptides of PS II and bacterial RCs also led to the suggestion that the different responses to bicarbonate might be due to the provision of the fifth and sixth ligands to the iron atom by Glu^{M234} in bacteria and the absence of this linkage in PS II; in PS II this role might then be filled by bicarbonate [9]. Direct interaction of some exogenous ligands with the iron atom of PS II is supported by EPR and Mössbauer studies, and modulation of these effects by HCO_3^- was interpreted as indicating that bicarbonate could be the fifth, and possibly the sixth, ligand to the non-haem iron of the acceptor quinone complex [10]. Some studies have implicated more than one site of action for HCO_3^- and a salt bridge function has also been postulated, as partner to an arginine [4]. We attempted to test the first proposal by replacing Glu^{M234} with valine, glutamine and glycine. All three mutants behaved similarly to the wild type and none exhibited a bicarbonate effect, implying that the absence of Glu^{M234} is not a sufficient requirement for this effect.

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Materials and Methods

Site-directed mutagenesis. Site-directed mutations of *Rb. sphaeroides* DNA were generated using two similar vector and expression systems described more fully elsewhere [11,12]. All constructions were based on the *Rb. sphaeroides* reaction center operon (*puf*), originally isolated as a 13 kb *Bam*HI fragment in pBR322 (pJW1; see Ref. 13). Deletion strains for expression of mutant genes from a plasmid were obtained through

double recombination with a suicide plasmid (pSUP202; see Ref. 14) containing the flanking DNA regions of the *Rb. sphaeroides puf* operon, with the operon, itself, replaced by a kanamycin resistance cassette (Km). The vectors for expression of mutant genes were based on pRK404, using either a 5.3 kb *Bam*HI/*Hind*III fragment [11] containing the *Rb. sphaeroides puf* operon, or a 4.5 kb *Pst*I fragment functioning under the *Tc*^r promoter of pRK404 [12]. Site directed mutagenesis was performed by standard techniques [15,16] either on the entire expression fragment or on smaller subclones. After sequencing to select and confirm the mutations, the expression fragments were cloned into pRK404 (carrying the tetracycline resistance marker, *Tc*^r) and transferred into a deletion strain of *Rb. sphaeroides* by diparental conjugation with the mobilizing strain of *E. coli*, S17-1 [14,17]. In one case, for the mutation Glu^{M234} → Val, the mutated gene was also incorporated into the genome by double recombination with the expression fragment in the suicide vector, pSUP202. Successful exconjugants were selected as *Ps*⁺ *Km*^s *Tc*^s. Maintenance of the mutation even after photoheterotrophic growth was established by dot blot analysis. This is consistent with the very similar growth rates of the M234 mutants compared to the deletion strain complemented with wild-type reaction-center genes.

The four mutations at position 234 of the M-subunit are:

M234EG <i>in trans</i> :	Glu → Gly, single base change; parent strain: Ga
M234EV <i>in cis</i> :	Glu → Val, two base change; parent strain: ATCC 17023
M234EV <i>in trans</i> :	Glu → Val, single base change; parent strain: ATCC 17023
M234EQ <i>in trans</i> :	Glu → Gln, single base change; parent strains: Ga and ATCC 17023

Bacterial culture. *Rb. sphaeroides* cells were grown in Sistrom's minimal medium [18] supplemented with 0.2% casamino acids. Kanamycin (25 µg/ml) and tetracycline (2 µg/ml) were present for the *in trans* mutants. 50 ml cultures were grown aerobically, in the dark, by vigorous shaking at 30°C, and then inoculated into 2 litres of medium for continued growth under semi-aerobic conditions to induce pigmentation and synthesis of photosynthetic components, including reaction centers. In some cases, the semiaerobic cultures were inoculated into 9.5 litres of Sistrom's medium, and grown under light for 4 days before harvesting. Sequencing of the M-subunit gene in plasmids isolated from light grown cells showed that there was no primary site reversion or any secondary site mutations within 150 bases of the M234 codon.

Chromatophore and reaction center preparation. Chromatophores, for *in situ* optical measurements, were prepared from *Rb. sphaeroides* strain Ga, and

from the various mutants, as previously described [19]. Reaction centers were prepared from *Rb. sphaeroides* strain ATCC 17023, strain Ga and the various mutants derived from these, by detergent fractionation of chromatophores with lauryldimethylamine *N*-oxide (LDAO). RCs were further purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation and by DEAE-Sephacel ion exchange chromatography [19,20]. Secondary acceptor activity was normally reduced to about 0.3–0.5 Q_B per RC. When desired, supplemental ubiquinone (20 μM) was added from a 20 mM stock suspended in 30% Triton X-100.

Optical measurements. Kinetic absorbance measurement in the microseconds to seconds time range were performed on an unchopped spectrophotometer of local design [20], with excitation provided by a xenon flash lamp (8 μs fwhh). Measurements of picosecond kinetics were performed on the apparatus described by Holzapfel et al. [21]. The excitation pulse width was less than 200 fs.

EPR measurements. Low-temperature EPR spectra were obtained using a Bruker 200D EPR spectrometer equipped with an Oxford Instruments Liquid Helium dewar system. Samples (0.3 ml of approx. 30 μM RCs)

were in 3 mm i.d. quartz tubes and were chemically reduced by the addition of 5 mM sodium dithionite immediately before freezing. Derivative spectra were obtained under field modulation (100 kHz) conditions.

Formate-bicarbonate treatment. Bicarbonate was depleted from the chromatophores and reaction center samples essentially as previously described [22]. Sodium formate was added to the sample to 100 mM at pH 6.0 and the sample degassed and flushed with CO_2 -free N_2 , prepared by passing N_2 gas through a column of CaCl_2 and ascarite before rehydrating it with distilled water. The bicarbonate depletion procedure was conducted at 25°C for at least 2 h to ensure complete depletion.

Results and Discussion

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

Following a flash, in the absence of secondary donors, the decay of P^+ in isolated RCs reflects recombination of the charge separated states, $P^+Q_A^-$ and $P^+Q_B^-$, depending on the reconstitution of Q_B function by the addition of ubiquinone. The decay of P^+ in the presence of ubiquinone occurs by recombination of

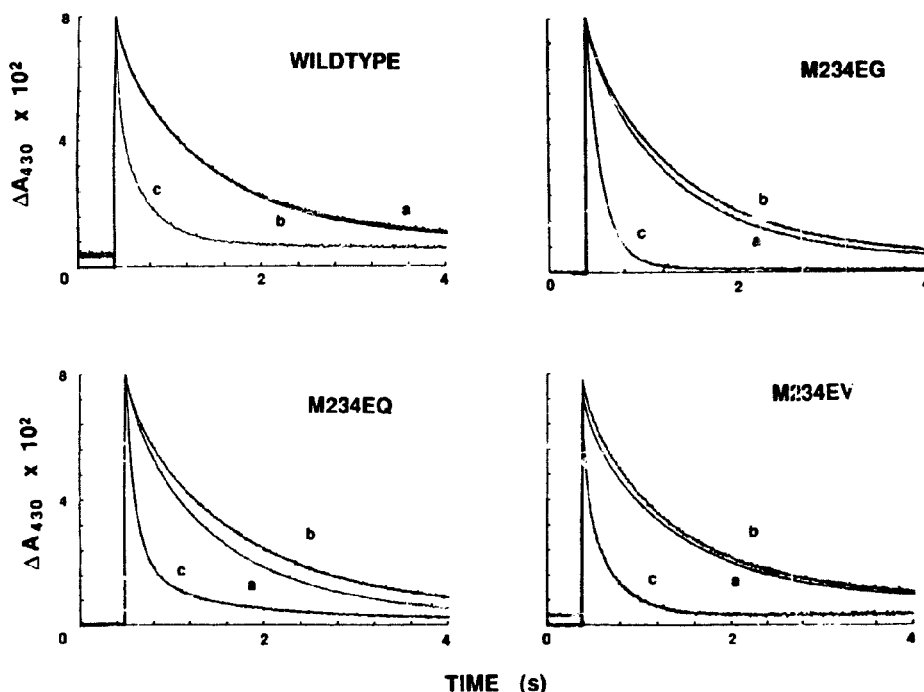
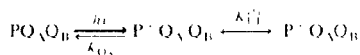


Fig. 1. $P^+Q_A^-$ and $P^+Q_B^-$ recombination kinetics in Wt and M234 mutant reaction centers. Recovery of the oxidized primary donor in the absence of exogenous donor, measured at 430 nm. Conditions: 2 μM RCs in 100 mM KCl and 10 mM Bistris at pH 6.0. 20 μM ubiquinone-10 was added with 0.03% Triton X-100. (a) No further additions; (b) bicarbonate depleted with 100 mM sodium formate and degassing at pH 6.0; (c) with 60 μM terbutryn. Wild type (Wt) and mutants M234EG, M234EQ and M234EV, as indicated.

$P^+Q_AQ_B$ through equilibrium with the P^+Q_A state, as follows [23,24]:



$K_{11}^{(1)}$ is the equilibrium constant for the one electron transfer between Q_A and Q_B and k_{QA} is the rate of recombination of P^+Q_A . Since the electron-transfer equilibrium is rapid compared to k_{QA} , the rate constant for recombination of the $P^+Q_B^-$ state is given by [23–25]:

$$k_{QB} = k_{QA}[1 + K_{11}^{(1)}]^{-1}$$

(Binding of quinone to the Q_B site is presumed saturated.) Thus, measurement of the rates of recombination of P^+Q_A (k_{QA} , determined in the absence of functional Q_B) and P^+Q_B (k_{QB} , determined in the presence of excess quinone) allows calculation of $K_{11}^{(1)}$.

Measurement of the charge recombination kinetics in isolated RCs showed very similar kinetics for the fast $P^+Q_A^-$ recombination or the slow $P^+Q_B^-$ back-reaction rate in Wt and M234 mutant PCs at pH 6.0 (Fig. 1),

and also at pH 8.0 and 10.0 (not shown). Furthermore, formate had no significant effect on these kinetics in any of the RC preparations (compare traces a and b). Bicarbonate (10 mM) itself had no effect (data not shown). Thus, neither the mutational changes nor formate affected the equilibrium sharing of an electron between the two quinones.

Even in the presence of added ubiquinone, a small amplitude of the fast phase of P^+ decay is seen in isolated RCs; this reflects RCs lacking Q_B , either because of incomplete saturation of the Q_B -binding site or because of damage leading to non-reconstitutibility [24,25]. The fraction of fast phase was similar in all preparations, ranging from 12% in Wt and M234EV mutant RCs to 20% in M234EG and, again, formate was without effect. Since 20 μ M Q-10 is sufficient to saturate the Q_B -binding site in Wt RCs [26], this fraction largely reflects non-reconstitutable RCs. Terbutryn, a competitive inhibitor of Q_B activity, strongly suppressed the slow phase of the back reaction in all preparations (see curves c in Fig. 1). Thus, the mutations did not markedly affect the binding or functional properties of the quinone sites.

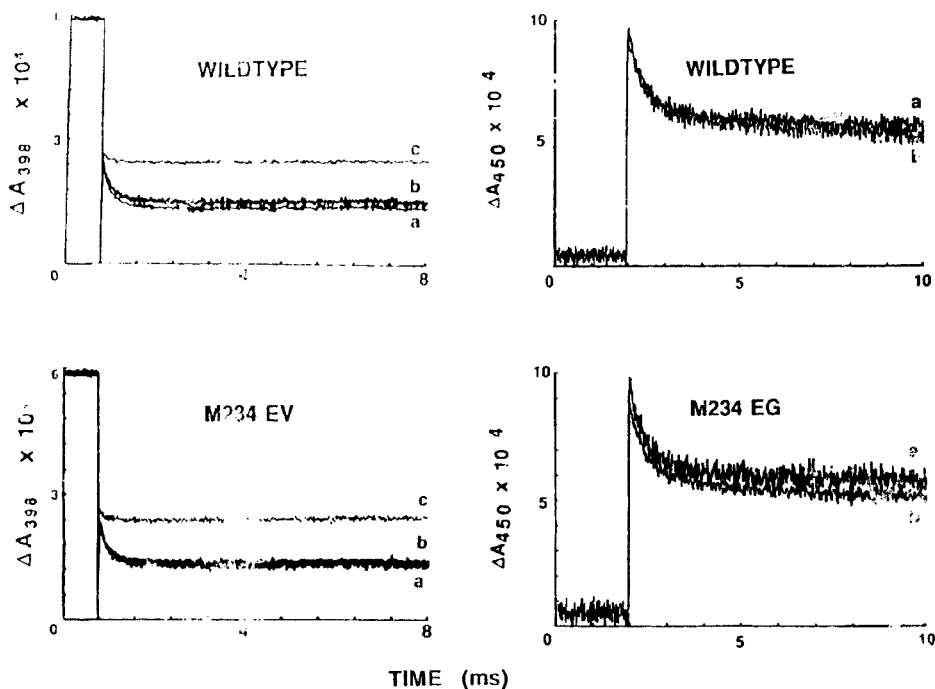


Fig. 2. Kinetics of electron transfer from Q_A to Q_B . Left: Q_AQ_B to $Q_AQ_B^-$ electron transfer kinetics in Wt (top) and M234EV (bottom) mutant reaction centers, measured at 398 nm. Conditions: as for Fig. 1. (a) No further addition; (b) bicarbonate depleted with 100 mM sodium formate and degassed at pH 6.0; (c) plus 60 μ M terbutryn. Right: Q_AQ_B to $Q_AQ_BH_2$ electron transfer kinetics in Wt (top) and M234EG (bottom) mutant reaction centers, measured at 450 nm after the second of two flashes given 1 s apart. Conditions: 2 μ M RCs in 100 mM KCl and 10 mM Mops at pH 7.0, 20 μ M ubiquinone-10, 0.63% Triton X-100, 10 μ M ferrocene. (a) No further addition; (b) bicarbonate depleted with 100 mM sodium formate and degassed at pH 6.0.

Direct measurement of electron transfer from Q_A to Q_B and from Q_A^- to Q_B

Transfer of the first electron from Q_A to Q_B , following a single flash, was detected by measuring the absorbance change at 398 nm. The kinetics for Wt and M234EV mutant RCs, at pH 6.0, are shown in Fig. 2 (left panels); identical results were obtained for M234EG and M234EQ mutant RCs. The instantaneous absorbance change is due to $P^+Q_A^-$. In Wt RCs, the subsequent electron transfer from Q_A^- to Q_B occurred with a half time of about 150–170 μ s when analyzed as a single exponential component. In all the M234 mutant RCs, the half-time was similar (170–180 μ s). Treatment of Wt or mutant RCs with 100 mM formate and flushing with N_2 for about 2 h at pH 6.0 did not change the kinetics of electron transfer from Q_A to Q_B (compare traces a and b). The inhibitor terbutryn effectively blocked electron transfer in all RC preparations (see curves c). Bicarbonate (10 mM) had no effect (data not shown). The Wt and all three M234 mutant RCs also exhibited similar electron transfer kinetics at pH 8.0 (not shown).

The kinetics of electron transfer from Q_A^- to Q_B after the second flash, were determined in the presence of a low concentration of ferrocene, sufficient to trap $PQ_A^-Q_B^-$ after the first flash. Following a second flash, the electron transfer from Q_A^- to Q_B was seen as a rapid disappearance of the semiquinone absorbance

signal at 450 nm (Fig. 2, right panels), preceding the decay of P^+ due to slow electron donation from ferrocene. For Wt RCs, at pH 7, the half time for electron transfer was 270 μ s. In M234EG and M234EV mutant RCs, the half-time was 330 μ s. Formate treatment had no consistent effect ($\leq 15\%$ change) on any preparation, and bicarbonate (10 mM) had no effect (not shown).

It is evident from these results that neither the first nor the second electron transfer to Q_B was kinetically impaired, or even detectably altered, in the M234 mutant RCs.

Two-electron gate oscillations of semiquinone formation and disappearance

In PS II and the purple bacteria, export of reducing equivalents is achieved through the acceptor quinone complex where the second quinone (Q_B) functions as a two-electron gate, passing reducing equivalents out of the RC only in pairs [27].

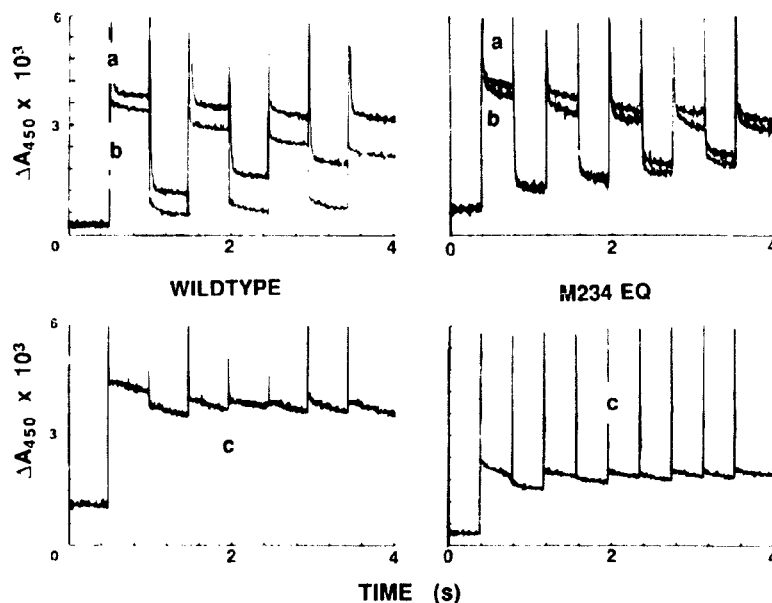
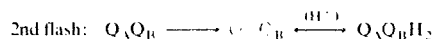
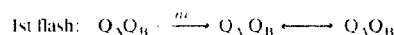


Fig. 3. Semiquinone oscillations measured at 450 nm. Conditions: 2 μ M RCs in 100 mM KCl, 10 mM Tris, 10 mM Bis-tris, pH 6.0, 20 μ M ubiquinone-10, 0.1% Triton X-100, and 500 μ M ferrocene. (a) No further additions; (b) bicarbonate depleted by addition of 100 mM sodium formate and degassed at pH 6.0; (c) plus 60 μ M terbutryn. Left panels: Wt RCs; right panels: M234EQ mutant RCs. Flashes were given at 1 s intervals.

(P^+ is re-reduced after each flash and is omitted from this scheme.) Q represents the quinone pool of the electron transport chain. Binary oscillations in the formation and disappearance of the stable anionic semiquinone of Q_B are shown in Fig. 3 (top panels). A stable semiquinone signal is observed after an odd number of flashes, giving an oscillation with a period of two (measured at 450 nm). The electron transfer equilibria between Q_A and Q_B generate a small population of Q_A^- , in which state the RC fails to turnover upon subsequent activation. This leads to damping of the oscillations [23,28]. Very similar oscillatory patterns were seen for Wt and M234EQ mutant RCs, and formate treatment did not affect the oscillation of the semiquinone signal in either type. The differences between traces a and b are due to some dilution during the formate treatment, and to baseline drifts due to differences in light scattering of the two samples. They do not reflect a significant change in the functional parameters of the oscillatory activity. This result indicates that the mutational change at M234 did not alter either the first or the second electron transfer equilibrium between Q_A and Q_B , or the yield of quinone reduction. Oscillations in the semiquinone signal were abolished by 60 μ M terbutryn in all samples (Fig. 3, bottom panels).

Electron flow in chromatophores

In *Rb. sphaeroides*, light-induced charge separation in reaction centers is followed, in situ, by a cyclic electron transfer from Q_B^- back to P^+ via the ubiquinone pool and cytochrome b/c_1 complex in the membrane, and cytochrome c_2 in the periplasm. Electron transfer to the b/c_1 complex was determined in Wt and M234EV mutant chromatophores by following the reduction of cytochrome b -561, measured from the absorbance difference between 561 and 574 nm (Fig. 4). Valinomycin was present to eliminate electrochromic shifts associated with the membrane electrical potential, and antimycin A was added to block the rapid reoxidation of b -type cytochromes. Ferricyanide was added in an attempt to fully oxidize Q_B , but this component is notoriously difficult to equilibrate [29]. The fact that cytochrome b reduction was observed after a single flash indicates that Q_B was not equilibrated with the prevailing redox potential, but was present in a roughly 50:50 mixture of Q_B and Q_B^- , essentially the steady-state condition. The extent and kinetics of reduction of cytochrome b -561 were the same in both Wt and M234EV mutant samples, and showed no sensitivity to formate treatment or to addition of bicarbonate (10 mM, not shown) in either Wt or mutant chromatophores. However, terbutryn was inhibitory, as expected (not shown). At the high ambient redox potentials used here, the kinetics of cytochrome b -561 reduction are very slow and non-exponential due

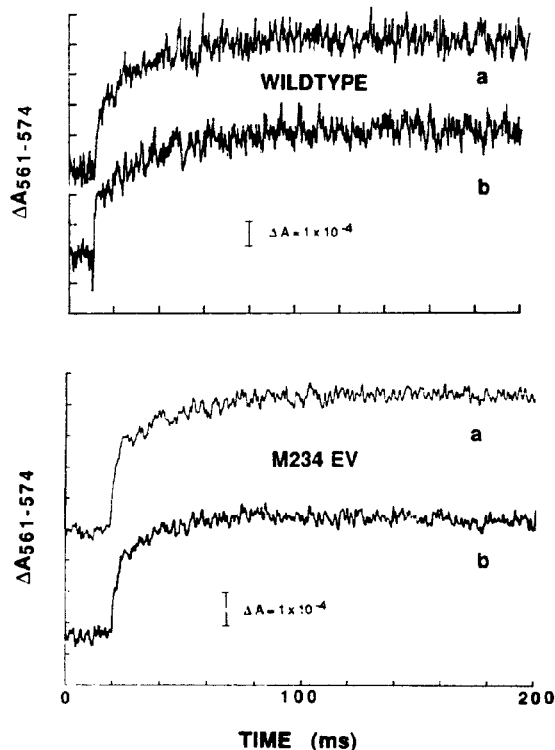


Fig. 4. Reduction of cytochrome b_{561} in chromatophores, measured by the absorbance change at 561-minus-574 nm. Chromatophores were added to 100 mM KCl, 10 mM Tris and 10 mM Bistris (pH 6.0) to an absorbance of 2 at 800 nm. Ferricyanide ($\approx 100 \mu$ M) was added to raise the redox potential to 380–400 mV, and 2 μ g/ml valinomycin and 2 μ g/ml antimycin A were also present. (a) No further addition; (b) bicarbonate depleted with addition of 100 mM sodium formate, and degassed at pH 6. Top panel: Wt chromatophores; bottom panel: M234EV mutant chromatophores.

to the second-order nature of the reaction [30,31]. The process involves the reduction and release of quinol from the reaction centers and diffusion of quinol to the cytochrome b/c_1 complex, with an overall half-time of 10–20 ms. The essential identity of the Wt and mutant kinetics strongly indicates that all potentially rate-limiting steps are unaltered in the mutants.

EPR signals of the Q_A semiquinone

The electron acceptor quinone complex of bacterial and PS II RCs is centered on an iron atom. Although the iron probably does not play an active redox role in electron transfer, it does exchange couple with the semiquinone species of Q_A and Q_B to give rise to unusual and very distinctive low-temperature EPR signals for $Q_A^--Fe^{II}$ and $Q_B^--Fe^{II}$, centered at $g = 1.82$ [32,33]. It is reasonable to expect these signals to be sensitive to the structural integrity of the acceptor quinone complex. The low temperature EPR signals

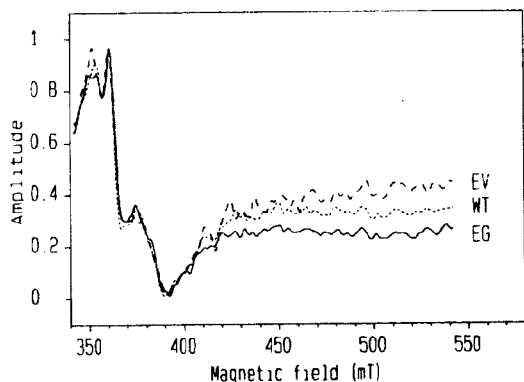


Fig. 5. Low-temperature EPR spectra of $Q_A\text{-Fe}^{\text{II}}$ in Wt and M234 mutant RCs. Samples containing 30 μM RCs, in 100 mM KCl, 10 mM Tris (pH 8.0), were reduced with 5 mM dithionite, immediately before freezing. Spectrometer settings: microwave power = 20 mW; frequency = 9.300 ± 0.002 GHz; modulation amplitude = 20 G; temperature = 5 K. Dotted line: Wt RCs; dashed line: M234EV mutant RCs; solid line: M234EG mutant RCs.

for $Q_A\text{-Fe}^{\text{II}}$ in Wt, M234EV and M234EG RCs, reduced by dithionite at pH 8.0, are shown in Fig. 5. Similar results were obtained at pH 6.0 and 10.0. There is no appreciable difference between Wt and the M234 mutant RCs, implying a remarkable conservation of the coupling and structural parameters of the interaction.

Primary events

The non-essentiality of the iron atom in acceptor quinone function has been demonstrated by the elegant extraction and reconstitution experiments of Debus et al. [34]. Removal of the iron, with careful retention of the H-subunit, caused only a 2-fold slowing of the first electron transfer to Q_B and a 3-4 fold slower steady-state turnover rate. The latter includes a significant decrease in the quantum yield of Q_A reduction which later measurements showed to be due to an unexpectedly large effect on the rate of reduction of Q_A by BPh^- , which was slowed from 200 ps to 4 ns [35]. Sub-nanosecond measurements on M234 mutant RCs showed no effect of the mutations on the very early events leading to reduction of bacteriophoto-

phytin, H_A , which have recently been resolved into two phases reflecting reduction of the accessory bacteriochlorophyll, B_A , in 3.5 ps, followed by electron transfer to H_A , with a characteristic time of 0.9 ps [21]. However, the subsequent electron transfer to Q_A was 2-fold slower in M234EQ and M234EV mutant RCs than in Wt RCs (Table I).

Replacing glutamate with glutamine, glycine or valine must eliminate one or more of the native ligands contributed from Glu^{M234} to the iron, as well as the partial compensation of the ferrous ion charge provided by an ionized glutamate. It would seem reasonable to expect, therefore, possibly significant structural alterations in the mutants – either to obtain suitable charge compensation and liganding from other nearby candidates (there are several acidic groups in this region of the structure) or to eliminate the iron atom altogether. However, the EPR signals of the Q_A semiquinone in Wt and mutant RCs indicate that any perturbations of the acceptor quinone complex do not affect the exchange coupling through the intervening histidine ligands. This result is partially confounded by the mild, but definite, effect of the mutations on the picosecond kinetics of electron transfer from BPh^- to Q_A . It is difficult to see, in a simple model, how this can be compatible with no perturbation of the $Q_A\text{-Fe}^{\text{II}}$ EPR signal, which should be a rather sensitive probe of both electrostatic and structural influences [33]. In PS II, for example, formate-induced depletion of bicarbonate causes a marked narrowing and intensification of this signal, although it is not affected by some herbicide resistance mutations in the Q_B pocket [36].

Conclusions

Studies on the bicarbonate effect in oxygen-evolving organisms have suggested two roles, possibly associated with two distinct binding sites [1–4,10]: (1) as a direct ligand with Fe^{II} , to provide a stable conformation for functional electron transfer; (2) as a proton donor to the plastoquinone reductase site in the D1-D2 protein. The kinetic behavior of Wt and Glu^{M234} mutant RCs of *Rb. sphaeroides* is summarized in Table I, which lists the lifetimes for all electron transfer steps involved in

TABLE I

Lifetimes (τ) for various electron transfer steps in reaction centers of Wt and M234 mutants

	$P^+ Q_A$ recombination	$P^+ Q_B$	$Q_A \rightarrow Q_B$ (μs)	$Q_A \rightarrow Q_B$ (μs)	$P^+ \rightarrow B_A$ (ps)	$B_A \rightarrow H_A$ (ps)	$H_A \rightarrow Q_A$ (ps)
	ms	ms					
Wt	170 ± 15	1125 ± 30	225 ± 20	390 ± 30	3.5 ± 0.4	0.9 ± 0.3	220 ± 20
M234EQ	165 ± 15	1095 ± 30	260 ± 20	–	3.5 ± 0.4	0.9 ± 0.3	410 ± 20
M234EV	155 ± 15	1090 ± 30	245 ± 20	460 ± 40	3.5 ± 0.4	0.9 ± 0.3	470 ± 20
M234EG	165 ± 15	1170 ± 30	245 ± 20	490 ± 40	–	–	–

the turnover of the reaction center. The mutants showed almost wild-type behavior for all processes and exhibited no effect of bicarbonate-depletion (formate treatment) on electron transfer from Q_A^- to Q_B and from Q_A to Q_B , on the two electron gate operation, or on the decay of the P^+Q_A and P^+Q_B states. Experiments on chromatophores from wild-type *Rh. sphaeroides* (Strain Ga) and from mutant M234EV showed no significant formate/bicarbonate effect on the light-initiated electron transfer from Q_A^- to cytochrome *b*-561. Furthermore, the EPR signal of the $Q_A^-Fe^{II}$ spin system suggests that any perturbations that may accompany the mutational substitutions at M234 are very small. These results imply that the structural integrity and function of the acceptor quinone complex in bacterial RCs, including the central iron-histidine ligand interaction, is not very sensitive to the provision of the fifth and sixth ligands by Glu^{M234} . It seems, therefore, that the requirement for bicarbonate in PS II, is not accounted for by a simple bicarbonate/carboxylate Fe-ligand homology, although it must be recognized that there are other primary structure differences between PS II and bacterial RCs, in this region. A possible involvement in proton transfer is not addressed directly as Glu^{M234} is not implicated in such an activity in bacterial RCs. However, recent site-directed mutagenesis studies on other carboxylic residues in the Q_B binding site of *Rh. sphaeroides* RCs have revealed a crucial role for Glu^{1212} and Asp^{1213} in proton transfer activities, and mutational lesions at these sites exhibited behavior reminiscent of bicarbonate-depleted activities in PS II [37]. The relationship between these observations is under further study.

Acknowledgements

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