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, QA and QB. 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 to valine, glutamine and glycine to form mutants M234EV, M234EO 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, M234EO 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+QA or P+QB, respectively; (b) the kinetics of electron transfer from QA to QB; or (c) the flash dependent oscillations of semiquinone formation in the presence of donor to P+ (QB 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 QA. The kinetics were resolved to reveal the reduction of the monomer bacteriochlorophyll (τ = 3.5 ps), followed by reduction of the bacteriopheophytin (τ = 0.9 ps). Both steps were essentially unaltered from the wild type. However, the rate of reduction of QA was slowed by a factor of 2 (τ = 410 ± 30 and 47 ± 30 ps for M234EO 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 QA 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 QA, the primary quinone, to QB, the secondary quinone acceptor of PS II [2]. It is considered that bicarbonate rather than CO2 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 QA to QB, a more marked inhibition of the second electron transfer from QA to QB and a
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 (RC) or in chromatophores of Rhodobacter sphaeroides [5]. The X-ray crystallographic structures of RCs from Rhodopseudomonas viridis [6] and Rhb. 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 Rhb. 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 *Rh. sphaeroides* DNA were generated using two similar vector and expression systems described more fully elsewhere [11,12]. All constructions were based on the *Rh. sphaeroides* reaction center operon (*puf*), originally isolated as a 13 kb *BamHl* 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 *Rh. 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 *BamHl/HindIII* fragment [11] containing the *Rh. sphaeroides* *puf* operon, or a 4.5 kb *PstI* fragment functioning under the Tc 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') and transferred into a deletion strain of *Rh. 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" Tc'. Maintenance of the mutation even after phototrophic 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 in trans**: Glu → Gly, single base change; parent strain: Ga
- **M234EV in cis**: Glu → Val, two base change; parent strain: ATCC 17023
- **M234EV in trans**: Glu → Val, single base change; parent strain: ATCC 17023
- **M234EQ in trans**: Glu → Gln, single base change; parent strains: Ga and ATCC 17023

**Bacterial culture.** *Rh. 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 *Rh. 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 (NH₄)₂SO₄ precipitation and by DEAE-Sephacel ion exchange chromatography [19,20]. Secondary acceptor activity was normally reduced to about 0.3-0.5 Q₅ 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 measurements 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 fwhm). 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 (10 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 CO₂-free N₂, prepared by passing N₂ gas through a column of CaCl₂ 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 'Q₅₋ and P 'Q₇₋ Following a flash, in the absence of secondary donors, the decay of P ' in isolated RCs reflects recombination of the charge separated states, P 'Q₅₋ and P 'Q₇₋, depending on the reconstitution of Q₇ function by the addition of ubiquinone. The decay of P ' in the presence of ubiquinone occurs by recombination of

![Graphs showing charge recombination kinetics](image-url)
$P^+ Q_h Q_h$ through equilibrium with the $P^+ Q_A$ state, as follows [23,24]:

$$P_h Q_h \xrightleftharpoons{K_{11}^{-1}} P^+ Q_h Q_h \xrightarrow{k_{Q_h}} P^+ Q_h H_2$$

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

$$k_{Q_h} = k_{Q_h} \left[1 + K_{11}^{-1} \right]^{-1}$$

(Binding of quinone to the $Q_h$ site is presumed saturated.) Thus, measurement of the rates of recombination of $P^+ Q_A$ ($k_{Q_h}$ determined in the absence of functional $Q_h$) and $P^+ Q_h$ ($k_{Q_h}$ 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 $P^+ Q_h$ back-reaction rate in Wt and M2344 mutant RCs 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 (19 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 $Q_h$, either because of incomplete saturation of the $Q_h$-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 μM Q-10 is sufficient to saturate the $Q_h$-binding site in Wt RCs [26], this fraction largely reflects non-reconstitutable RCs. Terbutryn, a competitive inhibitor of $Q_h$ activity, strongly suppressed the slow phase of the back reaction in all preparations (see curves e in Fig. 1). Thus, the mutations did not markedly affect the binding or functional proper ies of the quinone sites.

![Fig. 2. Kinetics of electron transfer from $Q_A$ to $Q_h$. Left: $Q_A Q_h Q_h$ to $Q_h Q_h$ 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 μM terbutryn. Right: $Q_A Q_h$ to $Q_h Q_h H_2$ electron transfer kinetics in Wt (top) and M234EV (bottom) mutant reaction centers, measured at 450 nm after the second of two flashes given 1 s apart. Conditions: 2 μM RCs in 100 mM KCl and 10 mM Mops at pH 7.0. 20 μM ubiquinone-10, 16% Triton X-100, 10 μM ferrocene. (a) No further addition; (b) bicarbonate depleted with 100 mM sodium formate and degassed at pH 6.0.](image-url)
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 absorption 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 $\mu$s when analyzed as a single exponential component. In all the M234 mutant RCs, the half-time was similar (170-180 $\mu$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 tert-butryn 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 $PQ_AQ_B$ 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 $\mu$s. In M234EG and M234EV mutant RCs, the half-time was 330 $\mu$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].

1st flash: $Q_AQ_B \rightarrow Q_AQ_B \rightarrow O_iQ_B$

2nd flash: $Q_AQ_B \rightarrow Q_AQ_B \rightarrow O_iQ_B$
(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$_h$ 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 equi-
libria 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 be-
tween 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 indi-
cates that the mutational change at M234 did not alter
either the first or the second electron transfer equilib-
rium between Q$_A$ and Q$_B$-, or the yield of quinone
reduction. Oscillations in the semiquinone signal were
abolished by 60 µM terbutryn in all samples (Fig. 3,
bottom panels).

**Electron flow in chromatophores**

In *Rh. 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. Elec-
tron 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 elec-
trochromic shifts associated with the membrane electri-
cal 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 equili-
ibrated with the prevailing redox potential, but was
present in a roughly 50:50 mixture of Q$_B^-$ and Q$_B^+$,
especially the steady-state condition. The extant 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 addi-
tion of bicarbonate (10 mM, not shown) in either Wt or
mutant chromatophores. However, terbutryn was in-
hibitory, 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
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 halftime of
10–20 ms. The essential identity of the Wt and mutant
kinetics strongly indicates that all potentially rate-limit-
ing 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 sig-
als 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
for $Q_\lambda$-$Fe^{II}$ in Wt, M234EV and M234EG RCs, reduced by dithionite at pH 8.0, and 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 Debuss 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 bacteriochlorophyll, $H_A$, 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 $Q_A$-$Fe^{II}$ 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 in 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 1, which lists the lifetimes for all electron transfer steps involved in

**Table I**

<table>
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<tr>
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<th>$P^+Q_A$ recombination</th>
<th>$P^+Q_B$ recombination</th>
<th>$O_A \rightarrow O_B$ (ps)</th>
<th>$Q_A \rightarrow O_B$ (ps)</th>
<th>$P^+H_A$ (ps)</th>
<th>$B_A \rightarrow H_A$ (ps)</th>
<th>$H_A \rightarrow Q_A$ (ps)</th>
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<tr>
<td>Wt</td>
<td>170 ± 15</td>
<td>1125 ± 30</td>
<td>225 ± 20</td>
<td>360 ± 30</td>
<td>3.5 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>220 ± 20</td>
</tr>
<tr>
<td>M234EQ</td>
<td>165 ± 15</td>
<td>1095 ± 30</td>
<td>260 ± 20</td>
<td>360 ± 30</td>
<td>3.5 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>410 ± 20</td>
</tr>
<tr>
<td>M234EV</td>
<td>155 ± 15</td>
<td>1090 ± 30</td>
<td>245 ± 20</td>
<td>460 ± 40</td>
<td>3.5 ± 0.4</td>
<td>0.9 ± 0.3</td>
<td>470 ± 20</td>
</tr>
<tr>
<td>M234EG</td>
<td>165 ± 15</td>
<td>1170 ± 30</td>
<td>245 ± 20</td>
<td>490 ± 40</td>
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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 to Q and from Q to Q, on the two electron gate operation, or on the decay of the P Q and P Q states. Experiments on chromatophores from wild-type Rh. sphaeroides (Strain Gs) and from mutant M234EV showed no significant formate/bicarbonate effect on the light-initiated electron transfer from Q to cytochrome b-561. Furthermore, the EPR signal of the Q-Fe 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 GluM234. 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 GluM234 is not implicated in such an activity in bacterial RCs. However, recent site-directed mutagenesis studies on other carboxylic residues in the O binding site of Rh. sphaeroides RCs have revealed a crucial role for GluM234 and AspM213 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.

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References