Absence of a bicarbonate-depletion effect in electron transfer between quinones in chromatophores and reaction centers of Rhodobacter sphaeroides

R.J. Shopes *, D.J. Blubaugh **, C.A. Wraight and Govindjee

Department of Physiology and Biophysics and Department of Plant Biology, University of Illinois, Urbana, IL (U.S.A.)

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Higher plants, algae, and cyanobacteria are known to require bicarbonate ions for electron flow from the first stable electron acceptor quinone QA to the second electron acceptor quinone QB, and to the intersystem quinone pool. It has been suggested that in Photosystem II of oxygenic photosynthesis, bicarbonate ions function to maintain the reaction center in a proper conformation and, perhaps, to provide the protons needed to stabilize the semiquinone (Q\textsuperscript{-}). In this paper, we show that bicarbonate ions do not influence the electron flow, from the quinone QA to QB and beyond, in the photosynthetic bacterium Rhodobacter sphaeroides. No measurable effect of bicarbonate depletion, obtained by competition with formate, was observed on cytochrome b-561 reduction in chromatophores; on the flash-dependent oscillation of semiquinone formation in reaction centers; on electron transfer from QA to QB; or on either the fast or slow recovery of the oxidized primary donor (P\textsuperscript{+}) which reflects the P\textsuperscript{+}QA → PQ\textsc{a} or the P\textsuperscript{+}Q\textsc{b} → PQ\textsubscript{B} reaction. The lack of an observed effect in Rhodobacter sphaeroides in contrast to the effect seen in Photosystem II is suggested to be due to the amino-acid sequence differences between the reaction centers of the two systems.

The electron acceptor complex of the reaction center (RC) of Photosystem II (PS II) of oxygenic organisms (higher plants, algae and cyanobacteria) and of the purple photosynthetic bacteria consist of two quinones (QA and QB) and a non-heme iron between them [1,2]. Experiments on chloroplasts from higher plants (see, for example, Refs. 3–5), leaves from higher plants (Refs. 6 and 7; see also El-Shintinawy, F. and Govindjee, unpublished results) and cells and thylakoids from cyanobacteria [9] have shown that depletion of bicarbonate (or CO\textsubscript{2}) reversibly decelerates the electron flow from the PS II electron acceptor complex (QA-Fe-QB) to the intersystem quinone pool. The effect is manifested in the decay of QA to QA, and in the control of the operation of the two electron gate in a pH-dependent manner (see, for example, Refs. 5, 10, 11). Blubaugh and Govindjee [12] have shown that HCO\textsubscript{3}\textsuperscript{-}, not CO\textsubscript{3}\textsuperscript{2-}, is the active species in thylakoids from higher plants. Furthermore, the requirement of HCO\textsubscript{3}\textsuperscript{-} in the operation of PS II appears to require two binding sites [13], one of which is suggested to be at the non-heme iron (see, for example, Refs. 14, 15) in QA-Fe-QB complex and the other postulated site is an arginine in the PS II RC [15,16]. Very little experimental data on the bicarbonate or formate-induced depletion effect in photosynthetic bacteria are available. Beijer and Rutherford [17] mentioned that addition of 200 mM formate (pH 6.0), to deplete bicarbonate, had no effect on the EPR signal of QA Fe\textsuperscript{2+} in chromatophores from the photosynthetic bacterium Rhodospirillum rubrum, in contrast to the effect on an analogous signal in higher plant thylakoids [18]. We show here that bicarbonate depletion by formate treatment has no effect on several electron transfer reactions involving the QA-QB complex in reaction center and chromatophore preparations from Rhodobacter (Rb.) sphaeroides.

Reaction centers were prepared from Rb. sphaeroides (strain, R-26) by detergent fractionation of chromatophores.
phores with lauryldimethylamine N-oxide and further purified by precipitation with ammonium sulfate followed by DEAE-cellulose chromatography (Maroti and Wraight [19]; also see pp. 39–44 in Stein [20]). Chromatophores were prepared from strain Ga of *Rb. sphaeroides*, as described on pp. 37–38 in Ref. 20. Kinetic absorbance measurements were carried out on a home-built double-beam spectrometer (for a description, see pp. 50–60 in Ref. 20).

Terbutryn was obtained from Chemical Services, Inc. (West Chester, PA), and all other chemicals were from Fisher Scientific Corporation (Itasca, IL) or Sigma Chemical Company (St. Louis, MO). Supplemental ubiquinone (10–30 μM) was added, when desired, from a 10 to 20 mM stock suspended in 10% LDAO or 30% Triton X-100. Bicarbonate was depleted from the chromatophore and the reaction center samples essentially as previously described [13]. Sodium formate was added to the sample to 100 mM at pH 6.0, and the sample degassed, and then 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 23°C for at least an hour to ensure complete depletion.

**Electron flow in chromatophores.** In purple photosynthetic bacteria, unlike oxygenic organisms, light-induced charge separation in the reaction center complex (PQA⁺ + hν → P⁺QA) is followed by a cyclic electron flow from QA back to P⁺ via quinone in the membrane, other quinones and cytochromes in a cytochrome b/c complex and cytochrome c in the periplasm. The effectiveness of this light-driven electron flow from the reaction center to the cytochrome b/c complex was measured in chromatophores by following the reduction of cytochrome b-561. In these experiments, valinomycin was included in the reaction mixture to eliminate electrochromic shift associated with the membrane electrical potential (ΔΨ). Antimycin A was added to block the rapid reoxidation of the b-type cytochrome via the so-called 'Q' cycle (see, for example, Cramer and Crofts [21]). Cytochrome b-561 reduction, at pH 6.0, measured from the absorbance difference between 561 nm and 569 nm, showed no significant effect of bicarbonate-depletion compared to control chromatophores, but showed a dramatic decrease by herbicide treatment (Fig. 1). Similar results were obtained at pH 8.0 (data not shown). Bicarbonate depletion had no apparent effect on any of the steps from the charge separation to the reduction of cytochrome b-561. Strictly speaking, bicarbonate depletion had no effect on any rate-limiting steps leading to the production and release of ubiquinol. In contrast, Photosystem II is known to show a dramatic reduction in its electron flow in an analogous location in a similar pathway [3–5].

**Two electron gate; oscillations in semiquinone production in reaction centers.** QA is a one electron acceptor, whereas QB is a two electron acceptor. Electron flow can be written, omitting the details of protonation, as follows (see Wraight [22] and Verméglio [23]):

\[
 QA + Q_B \overset{k_1}{\rightarrow} QAQ_B \caption{(a)}
\]

\[
 QAQ_B \overset{k_2}{\rightarrow} QAQ_B + QH_2 \overset{2k}{\rightarrow} 2QA + QH_2 \caption{(b)}
\]
Here, $^1h\nu$ and $^2h\nu$ stand for 1st and 2nd flash, respectively. A stable semiquinone signal is observed only after an odd number of flashes, giving an oscillation with a period of two. Double hits and misses lead to a dampening of the oscillation. We tested the effect of bicarbonate-depletion (formate treatment) on the oscillation of semiquinone. In reaction center preparations containing excess ubiquinone, typical binary oscillation of the semiquinone formation (measured at 450 nm) was clearly observed at pH 8.0 (Fig. 2). Bicarbonate-depletion (formate treatment at pH 6.0) did not appreciably change these oscillations measured at pH 8.0. Similar results were obtained in measurements at pH 6.0 (data not shown). However, as expected (see Wraight [24]), the inhibitors o-phenanthroline and terbutryn effectively abolished the semiquinone oscillations as they compete with $Q_B$ and do not allow reaction 1 to occur; thus, electron transfer to $Q_B$ is blocked.

The lack of an effect of bicarbonate depletion on the semiquinone oscillations specifically indicates no changes in the factors relevant to the operation of the two electron gate. There are no changes in the yields of any of the quinone states, $Q_AQ_B$, $Q_AQ_B^-$, and $Q_AQ_B^{2-}$ (2H+) in *Rb. sphaeroides*, as opposed to its effect in PS II (see, for example, Refs. 10, 11, 25–27).

**Direct measurement of $Q_A^-$ to $Q_B$ reaction in reaction centers.** We have measured the $Q_A^-Q_B \rightarrow Q_AQ_B^-$ reaction by following the absorbance change at 398 nm. This wavelength is close to the isosbestic point for $P^+Q_A/PQ_A^-$. $P^+Q_A^-$ and $P^+Q_B$ have been found to have a slightly different absorbance spectra in this region (see p. 77 in Ref. 20; see also Ref. 23). Although, as expected, the inhibitors blocked the electron flow from $Q_A^-$ to $Q_B$ (as reflected in the absorbance change), bicarbonate-depletion (formate treatment) gave no effect on this reaction in the reaction centers from *Rb. sphaeroides* (Fig. 3). This is contrary to dramatic effects seen in PS II [10,11,26].

**Back reactions in reaction centers: fast ($P^+Q_A^- \rightarrow PQ_A$) and slow ($P^+Q_B \rightarrow PQ_B$).** Wraight [22] and Wraight and Stein [28] proposed that the observed back reaction (i.e., the recovery kinetics of $P$) could be described as follows:

$$
\begin{align*}
\text{Reaction Rate} &= k_{+} [PQ_A] \quad \text{for a fast reaction,} \\
&= k_{-} [PQ_B] \quad \text{for a slow reaction.}
\end{align*}
$$

A faster back reaction reflects $P^+Q_A^-$ to $PQ_A$ reaction, and a slower back reaction reflects $P^+Q_B$ to $PQ_B$ reaction. The ratio of the two rates reveals the equilibrium sharing of an electron between the two quinones (Stein et al. [29]). The back reaction kinetics reflects the one-electron equilibrium ($K = k_{-}/k_{-}^2$) between $Q_A$ and $Q_B$; it is given as:

$$
\begin{align*}
t_{1/2} (\text{observed}) &= t_{1/2} \left[ Q_A (1 + K) \right] \\
&= \frac{1}{k_{-}^2} [PQ_A] \\
&= \frac{1}{K} \frac{1}{k_{-}^2} [PQ_A] \\
&= \frac{1}{k_{-}^2} \left( 1 + \frac{k_{-}}{k_{+}} \right) [PQ_A]
\end{align*}
$$

where $t_{1/2}$ is the half-time for recombination when only $Q_A$ is functioning. Our measurements on the recovery of the primary donor (P) at 430 nm in reaction centers of *Rb. sphaeroides* at pH 7. (Fig. 4) revealed that the slow back reaction was unaffected upon formate treatment (bicarbonate depletion), whereas the inhibitors terbutryn and o-phenanthroline eliminated the slow back reaction, since they compete with $Q_B$ [24]. Similar
results were obtained at pH 6 and 8 (data not shown). In addition, the fast back reaction in the presence of inhibitors remained the same with or without formate addition. However, in PS II, the back reaction involving Q_B, detected by thermoluminescence measurements, decreases dramatically upon bicarbonate-depletion [7,30].

**Concluding remarks.** Our experiments on *Rb. sphaeroides* reaction centers show no effect of bicarbonate-depletion (formate treatment) on electron flow from Q_A to Q_B (Fig. 3); the two-electron gate operation (Fig. 2); or the decay of the P^+Q_B^- or the P^+Q_A^- state (Fig. 4). Furthermore, experiments with chromatophores from *Rb. sphaeroides* (strain Ga) reveal the absence of any significant bicarbonate effect on the light-initiated electron flow from Q_A to cytochrome b-561 (Fig. 1). These results, taken together with the absence of the formate effect on the EPR signal of Fe-quinone complex [17], show that electron transport through the quinones of photosynthetic bacteria is not affected by lack of bicarbonate. Furthermore, our unpublished data show that addition of even 100 mM...
bicarbonate (after correction for pH changes) had no effect on the quinone related reactions of photosynthetic bacteria. This contrasts with the data on all oxygenic organisms, including cyanobacteria. A possible reason for the difference may be in the difference in the architecture of the PS II and the photosynthetic bacterial reaction centers. For example, Michel and Deisenhofer [14] have suggested that HCO$_3^-$ may act as a ligand to the non-heme iron in PS II, and in photosynthetic bacteria glutamate-232 on the M-subunit may be the counterion instead. Notably, a small section of the amino acid sequence in this region, in an analogous subunit, is deleted in the PS II RC. Blubaugh and Govindjee [13,15] have suggested that, in plants, there are two cooperative HCO$_3^-$ binding sites. It remains to be seen whether cyanobacteria, which may be intermediate between photosynthetic bacteria and plants, have only one or two binding sites. Here, we have shown a lack of an effect in the bacterial photosynthetic electron transfer at the QA-Fe-QB level and beyond.

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References

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