Biochimica et Biophysica Acta, 974 (1989) 114–118
Elsevier

BBA 40222

BBA Report

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.)

(Received 28 September 1988)

Key words: Bicarbonate effect; Electron transfer; Quinone; Photosynthetic bacterium; Chromatophore; Reaction center; Electron acceptor complex; Photosynthesis; (*Rb. sphaeroides*)

Higher plants, algae, and cyanobacteria are known to require bicarbonate ions for electron flow from the first stable electron acceptor quinone Q_A to the second electron acceptor quinone Q_B, and to the intersystem quinone pool. It has been suggested that in Photosystem II of oxygenic photosynthesis, bicarbonate ion functions to maintain the reaction center in a proper conformation and, perhaps, to provide the protons needed to stabilize the semiquinone (Q_B^-). In this paper, we show that bicarbonate ions do not influence the electron flow, from the quinone Q_A to Q_B 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 Q_A^- to Q_B; or on either the fast or slow recovery of the oxidized primary donor (P^+) which reflects the $P^+Q_A^- \rightarrow PQ_A$ or the $P^+Q_B^- \rightarrow PQ_B$ 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 (Q_A and Q_B) 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(see, for example, Refs. 3–5), leaves from higher plants (Refs. 6 and 7; see also El-Shintinawy, F. and Govindjee, unpublished results) and cells and thylakoids from cyanobacteria [9] have shown that depletion of bicarbonate (or CO_2) reversibly decelerates the electron flow from the PS II electron acceptor complex (Q_A-Fe-Q_B) to the intersystem quinone pool. The effect is manifested in the decay of Q_A^- to Q_A, 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_3^-, not CO_2, is the active species in thylakoids from higher plants. Furthermore, the requirement of HCO_3^- 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 Q_A-Fe-Q_B 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 Q_A^- Fe^{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 Q_A-Q_B 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 chromato-

* Present address: Department of Cell Biology, Stanford University, Stanford, CA 94305, U.S.A.

** Present address: Department of Agronomy, University of Kentucky, N-212 Agricultural Sciences North, Lexington, KY 40546-0091, U.S.A.

Abbreviations: LDAO, lauryldimethylamine *N*-oxide; P, primary electron donor; PS II, Photosystem II; *Rb.*, *Rhodobacter*; RC, reaction center; Q_A, first bound quinone acceptor; Q_B, second bound quinone acceptor.

Correspondence: Govindjee, Department of Plant Biology, University of Illinois 289 Morrill Hall, 505 S. Goodwin Avenue, Urbana, IL 61801, U.S.A.

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 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 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 ($PQ_A + h\nu \rightarrow P^+Q_A^-$) is followed by a cyclic electron flow from Q_A^- 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 ($\Delta\psi$). 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. Q_A is a one electron acceptor,

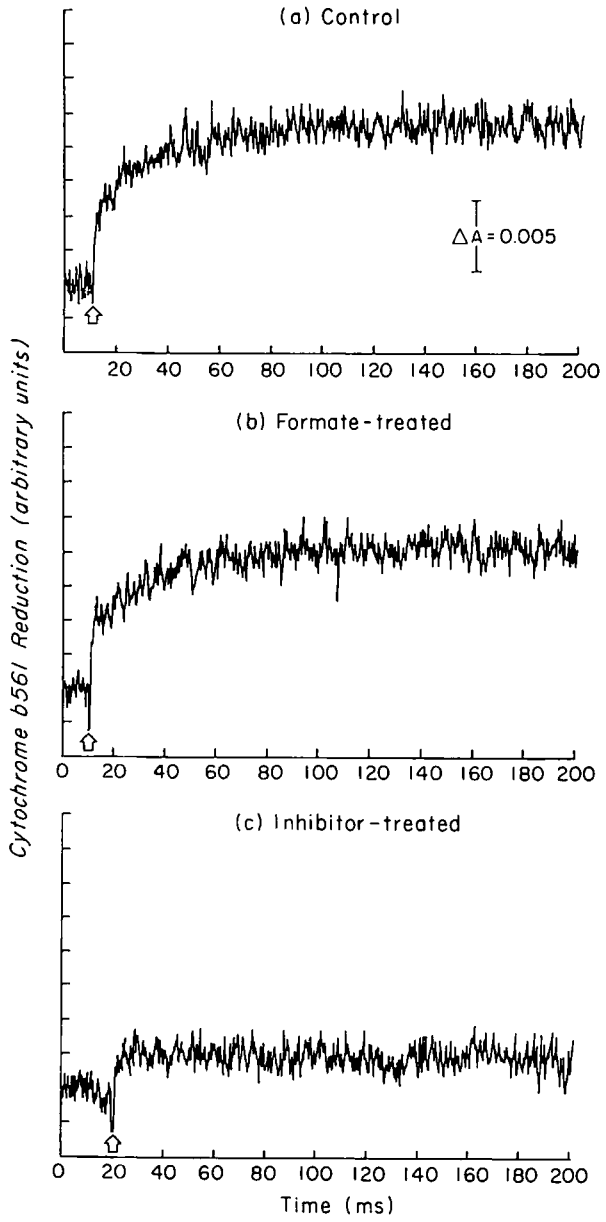

Fig. 1. Reduction of cytochrome *b*-561 in chromatophores was measured by absorbance change at 561 nm minus that at 569 nm. Chromatophores were added to 100 mM NaCl, 10 mM Tris and 10 mM Mes at pH 6 to an absorbance of 2 at 800 nm. Ferricyanide (100 μM) was added to raise the redox potential to 415 mV. 2 μg/ml valinomycin and antimycin A were added. (a) No further additions; (b) bicarbonate depleted with the addition of 100 mM sodium formate, and degassing at pH 6, as detailed in Materials and Methods; (c) 4 mM *o*-phenanthroline and 50 μM terbutryn were added. Arrows indicate exciting xenon flashes.

whereas Q_B is a two electron acceptor. Electron flow can be written, omitting the details of protonation, as follows (see Wraight [22] and Verméglio [23]):

$$Q_A \cdot Q_B \overset{^1h\nu}{\Longrightarrow} Q_A^- Q_B \rightleftarrows Q_A Q_B^- \qquad (1a)$$

$$Q_A Q_B^- \overset{^2h\nu}{\Longrightarrow} Q_A^- Q_B^- \rightleftarrows Q_A Q_B^{2-} \overset{Q \quad QH_2}{\underset{2H^+}{\dashrightarrow}} Q_A Q_B \qquad (1b)$$

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)

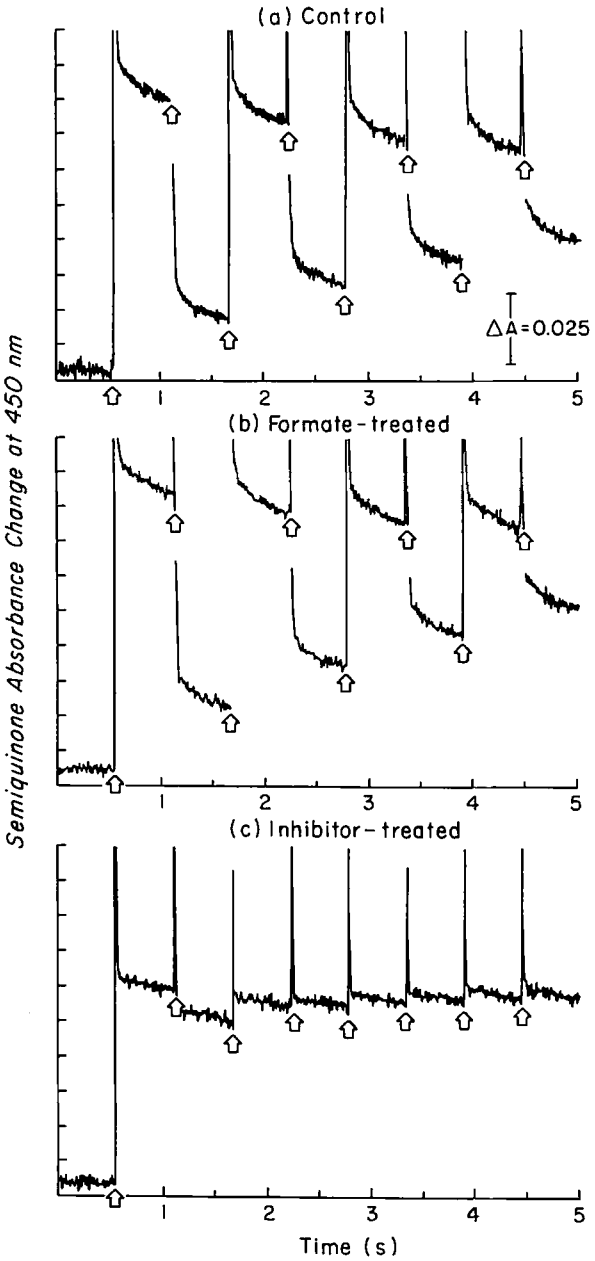

Fig. 2. Semiquinone oscillation was measured at 450 nm. Reaction centers (2 μM) were added to 100 mM NaCl and 10 mM Tris and 10 mM Mes at pH 8. 100 μM ubiquinone-10, 0.15% Triton X-100, and 100 μg/ml of DAD and TMPD were also added. The redox potential was 190 mV. (a) No further additions; (b) bicarbonate depleted with the addition of 100 mM sodium formate and degassing at pH 6; (c) with 4 mM o-phenanthroline and 50 μM terbutryn. Arrows indicate exciting xenon flashes (1–8).

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:

$$PQ_AQ_B \underset{k_0}{\overset{h\nu}{\rightleftharpoons}} P^+Q_A^-Q_B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} P^+Q_AQ_B^- \qquad (2a)$$

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 ($K_2 = k_2/k_{-2}$) between Q_A and Q_B; it is given as:

$$t_{1/2}\ (\text{observed}) = t_{1/2}Q_A(1 + K_2) \qquad (2b)$$

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

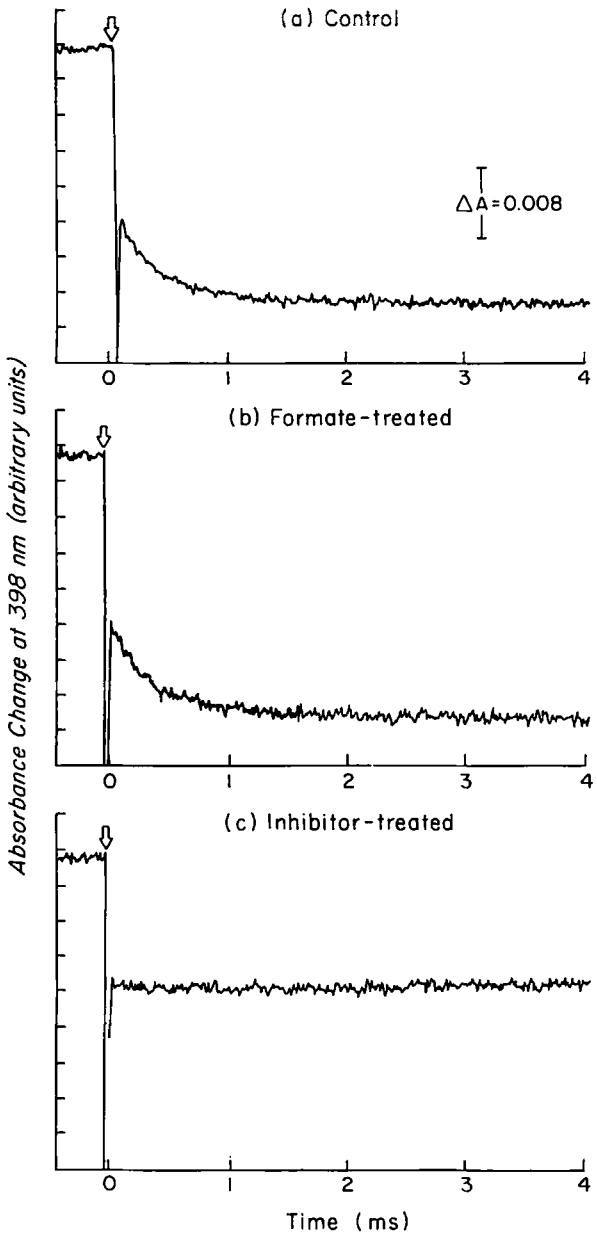

Fig. 3. $Q_A^- Q_B$ to $Q_A Q_B^-$ electron transfer was measured at 398 nm. Reaction centers (4 μM) were added to 100 mM NaCl and 10 mM Tris and 10 mM Mes at pH 7. 20 μM ubiquinone-10 was added to 0.03% Triton X-100. (a) No further additions; (b) bicarbonate depleted with the addition of 100 mM formate and degassing at pH 6, measured at pH 7.0; (c) addition of 4 mM *o*-phenanthroline and 50 μM terbutryn. (See Stein [20] for method.) Arrows indicate exciting xenon flashes.

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

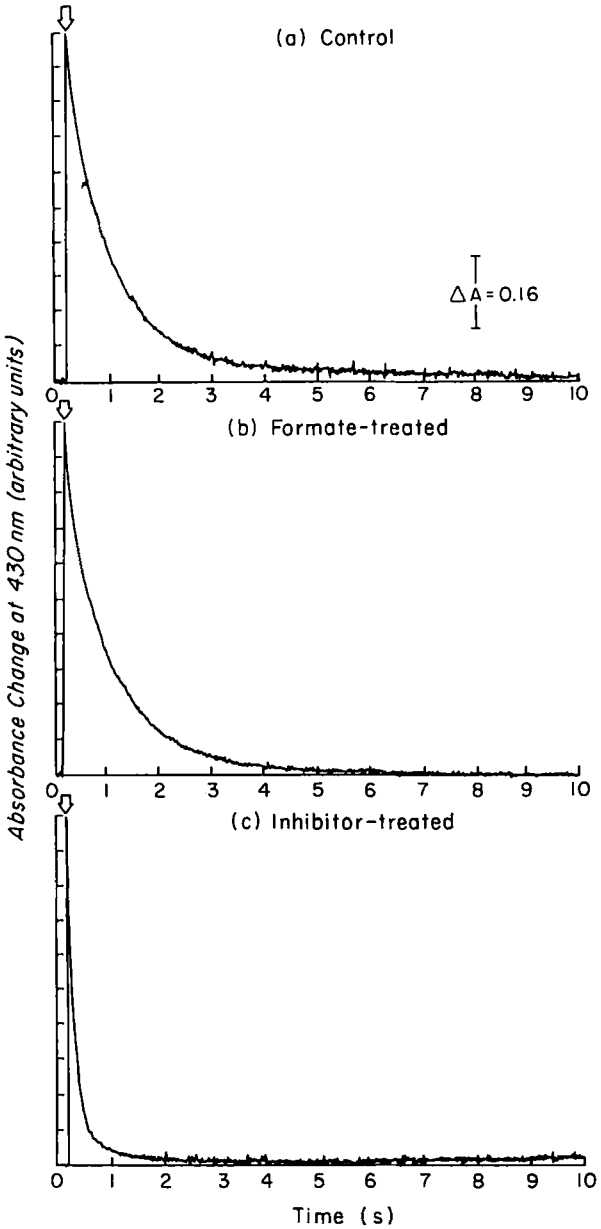

Fig. 4. Recovery of the oxidized primary donor was measured at 430 nm. Conditions were as in Fig. 3. (a) No further additions; (b) bicarbonate depleted with the addition of 100 nM sodium formate and degassing at pH 6 (see text); (c) with 4 mM *o*-phenanthroline and 50 *M* terbutryn. Arrows indicate exciting flashes.

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 Q_A-Fe-Q_B level and beyond.

R.J.S. and D.J.B. were supported by a training grant (PHS 5-T32GM7283) from the USPHS. Govindjee and C.A.W. were supported by NSF grants. We thank Dr. Peter Maroti for discussions, and Dr. Rita Khanna for the earliest measurements on this topic in 1978.

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