PHOTOSYSTEM II: FROM A FEMTOSECOND TO A MILLISECOND

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ABSTRACT

We present here a minireview on the time sequence of events in water-plastoquinone oxidoreductase (i.e., photosystem II, PSII) from a femtosecond to a millisecond. The minimal components of PSII include: three intrinsic polypeptides D-1 and D-2, and cytochrome (Cyt) b-559, and an extrinsic polypeptide of 33 kilodalton (kDa) mass (labeled as Y-33). The first act of photosynthesis, light absorption, occurs in chlorophyll (Chl) a molecules in about 1 femtosecond (fs). Then, the following reactions take place. (1) The primary charge separation, i.e., the formation of the oxidized reaction center Chl a, P680+, and the reduced pheophytin, Pheo−, has been shown to occur in reaction center preparation (Nanba and Satoh, 1987) with a lifetime of 3 ± 0.6 picoseconds (ps) (Wasielewski et al., 1989), using 500 fs laser flashes. (2) Electron flow from Pheo− to the first plastoquinone electron acceptor, Q_A, has been estimated to occur in several hundreds of ps. (3) The re-reduction of P680+ by the electron donor Z [suggested recently to be a tyrosine molecule (Debus et al., 1988; Vermaas et al., 1988, in press)] occurs in 25 to 300 nanoseconds (ns) (Bretet et al., 1984). (4) The electron flow from the reduced Q_A, Q_A−, to the second plastoquinone electron acceptor, Q_B, occurs in several hundredths of a microsecond (µs). Approximately, in the same time range, Z+ is restored to Z by electron flow from the water oxidation complex (WOC) producing the stable charge-separated state S_2Q_B−, where S_2 refers to the redox state of the WOC -- the dark-adapted state being S_1Q_B. Thus, after twelve orders of magnitude of time, a stable state is created. We shall not discuss here the steps of oxygen evolution, and the details of the production of plastoquinol. However, we shall review the possible role of bicarbonate (HCO₃⁻) in the mechanism of plastoquinone reduction in plants and cyanobacteria (Blubaugh and Govindjee, 1988, in press). It is suggested
that HCO$_3^-$ functions to provide (a) a proper conformation to the reaction center by binding to the iron in the $Q_A$-$Fe$-$Q_B$ complex, and (b) protons for plastoquinol formation by binding to an arginine/histidine pair on the D-1 protein of PSII.

INTRODUCTION

The function of Photosystem II (PSII) is to act as water-plastoquinone oxido-reductase (WPOR). Water is oxidized to O$_2$, and plastoquinone (PQ) is reduced to plastoquinol (PQH$_2$): $2$H$_2$O + 2PQ + 4H$^+$ (outside) + 4 h$^+$ $\rightarrow$ O$_2$ + 2 PQH$_2$ + 4H$^+$ (inside). This uphill transfer of electrons requires 4 light quanta (h$^+$), which are used via the excitation of the reaction center (RC) chlorophyll (Chl) $a$ of PSII, P680; the latter leads to the primary charge separation: P680 $\cdot$ Pheo + h$^+$ $\rightarrow$ P680$^+$ $\cdot$ Pheo $\rightarrow$ P680$^+$ $\cdot$ Pheo$^-$, where Pheo stands for pheophytin. This minireview is concerned solely with the light reaction and the electron transport in PSII. For earlier reviews, see Govindjee (1980, 1982, 1984), Velthuys (1980, 1987), van Gorkom (1985), Diner (1986), Mathis (1986), Ort (1986), and Andréasson and Vännégård (1988). The electron acceptor side of PSII has been discussed by Vermaas and Govindjee (1981), Crofts and Wraight (1983), and Govindjee and Eaton-Rye (1986), and the electron donor side by Ghanotakis and Yocum (1985), Govindjee et al. (1985), Renger and Govindjee (1985), Andersson and Åkerlund (1987), Babcock (1987), and Renger (1987).

Figure 1 shows a self-explanatory generalized model of the thylakoid membrane with the four major complexes: PSII, cytochrome b$_6$/f complex, PSI and the ATP synthase, whereas Fig. 2 shows a detailed current picture of the electron flow in PSII, along with the measured or estimated times of reactions. Electron carrier redox pairs are placed vertically according to their approximate known or estimated redox midpoint potential (Em,7). The main path of electron flow is as follows (alternate names of the intermediates are in the figure): H$_2$O/O$_2$ (Em,7, +0.82V) $\rightarrow$ Mn$^{3+}$/Mn$^{4+}$ $\rightarrow$ Z/Z$^+$ (+1.0V; tyrosine$^\cdot$/tyrosine$^+$) $\rightarrow$ P680/P680$^+$ (+1.15V) h$^+$ $\rightarrow$ P680/P680$^*$ (-0.7V) $\rightarrow$ Pheo/Pheo$^-$ (-0.6V) $\rightarrow$ Q$_A$/Q$_A^-$ (0 to -30mV) $\rightarrow$ Q$_B$/Q$_B^-$ $\rightarrow$ PQ/PQH$_2$ (+90mV). Oxygen and protons are released to the inside, and protons are picked up from the outside for the production of PQH$_2$. Here, the symbol "M" under Mn$^{3+}$/Mn$^{4+}$ represents more than the Mn atoms: it is the charge-accumulating entity necessary for water oxidation and it may, therefore, include the necessary (Y-33, the extrinsic polypeptide with 33 kDa molecular mass) and stimulatory (regulatory) polypeptides (Y-24 and Y-18), chloride (Cl$^-$), and entities (proteins?) producing absorbance changes at 320nm (Y-320), etc. The redox state of "M" is referred to as the S-states, where S$_0$, S$_1$, S$_2$, S$_3$, and S$_4$ represent the five different states.
with increasing positive equivalents on them. "Z" represents the entities that donate electrons directly to P680$^+$; the oxidized form of Z, Z$^+$, is described by its ESR signal labeled II very fast (IIv II); earlier, it was suggested to be an oxidized plastoquinol molecule. Now, new data suggest that it may be tyrosine-160 on the polypeptide D-1 of PSII (Debus et al., 1988, Vermaas et al., 1988). P680, which is most likely a dimer in its ground state, behaves as a monomer in its cationic and triplet form. In the current picture, it is attached to the α-helices of the D-1 and D-2 polypeptides of the reaction center, just as the reaction center bacteriochlorophyll is attached to the "L" and "M" subunits of the bacterial reaction center (Michel and Deisenhofer, 1988). The photochemically active Pheo, one of the two lpheophytin molecules in the reaction center complex, is a monomer and suggested to be located on the D-1 polypeptide (see Fig. 3). QA, the bound plastoquinone, may also be on D-2; by analogy, again, with photosynthetic bacteria, a non-heme iron (Fe$^{2+}$) sits in the middle of QA and the second bound plastoquinone QB, which is located on D-1. QB acts as a two-electron gate; it is very tightly

**Figure 1.** A schematic diagram of the four major protein complexes. From left to right: Photosystem II [OEC = oxygen evolving complex, P680 = reaction center chlorophyll a, Ph = functional pheophytin, QA = first plastoquinone electron acceptor (on D-2), QB = second plastoquinone electron acceptor (on D-1), PQ (PQH$^-$) = mobile plastoquinone (plastoquinol)]; Cytochrome $b_6/f$ complex (cyt $b_6$ = cytochrome $b_6$, Cyt $f$ = cytochrome $f$, FeS = Rieske Fe-S center, Ppc = plastocyanin); Photosystem I (P700 = reaction center chlorophyll a, Ao = chlorophyll a, first electron acceptor, FeS = iron sulfur centers, Fd = ferredoxin); and the ATP synthase (the membrane portion is called CF$_0$; and the hydrophilic portion is called CF$_1$). Simplified by Donald R. Ort (1988, personal communication) from Govindjee and Eaton-Rye (1986). Courtesy of D. Ort.
Figure 2. Electron transport scheme of Photosystem IIα. Ovals show major components; measured or estimated times involved in each step are also shown. Location of sites of Cl⁻ and HCO₃⁻ actions are highlighted. Alternate names of intermediates, used by various authors for the major components, are also shown (see text). Modified after Govindjee (1984) and Govindjee et al. (1985).

bound in its Q<sub>B</sub><sup>-</sup> form; and it exchanges with a plastoquinone molecule only after it is doubly reduced (Crofts and Wraight 1983; Velthuys, 1987). Both the D-1 and D-2 are suggested to span the membrane 5 times (Trebst and Draber, 1986; Trebst, 1987). Herbicides (Trebst 1986) and bicarbonate (Blubaugh and Govindjee 1988) are suggested to bind to this protein on the side facing the stroma, whereas manganese and chloride, involved in O₂ evolution, are suggested to bind on the side facing the lumen (Coleman and Govindjee, 1987).

A working model for the organization of PSI (excluding the light harvesting Chl a/Chl b complex) is shown in Fig. 4. In most higher plants, active PSI is located in the "appressed" regions of the membrane; this is, perhaps, the PSI IIα units (for a discussion of heterogeneity of PSI, see Vermaas and Govindjee, 1981; and Black et al., 1986). We recognize a minimum of 7 polypeptides: (I) 47 kDa (CP-47), an internal Chl a-protein antenna complex; it fluoresces at 696nm at 77K; (II) 43 kDa (CP-43), a distal Chl a-protein antenna complex; it fluoresces at 685nm at 77K; (III) D-1: 32 kDa Q<sub>B</sub><sup>-</sup>-binding lysine-rich protein to which herbicides can also bind (HBP) and that may also carry bicarbonate binding sites (BBP); (IV) D-2: 34 kDa Q<sub>A</sub><sup>-</sup>-binding lysine-poor protein; (V) cytchrome b-559 having two polypeptides of 4 and 9 kDa molecular masses; (VI) Y-33: a 33 kDa extrinsic polypeptide, that can be released by high concentrations (1M) of CaCl₂, is involved in O₂ evolution; (VII) Y-24: a 24 kDa extrinsic polypeptide that can be released by low
concentrations of salts; it stimulates $O_2$ evolution and is required for $Ca^{2+}$ and $Cl^-$ binding to the membrane; and (VIII) Y-18: a 18 kDa extrinsic polypeptide that can also be released by low concentrations of salts; its function is less clear than that of Y-24. Cyanobacteria do not contain Y-18 and Y-24. The minimal system required, thus far, for $O_2$ evolution contains, at least, the first 6 polypeptides (Ghanotakis et al., 1987). However, the reaction center (RC) preparation, capable of the primary, charge separation, is simpler and was shown to contain only three polypeptides: D-1, D-2, and cytochrome $b$-559; it contained 4 chlorophyll
molecules (2 of which, presumably, form P680) and 2 pheophytin molecules (Nanba and Satoh, 1987). Unfortunately, the currently available RC preparations lack $Q_A$ and $Q_B$. Although the role of cytochrome $b-559$ in the main electron flow has not been discovered, it has been shown to act as an electron donor at 77K or when normal-electron flow is blocked.

The major electrogenic event in PSII is suggested to be due to the electron flow from reduced pheophytin to $Q_A^-$. This is supported by electrically-induced reduction of Pheophytin by $Q_A^-$ (Meiburg et al., 1983); a direct kinetic measurement of membrane potential by a macroscopic electrode technique in PSII suggests that it is a fast reaction (<200 ps) (see Trissl and Kunze, 1985).

What follows is a discussion of the major components and their function in the electron transfer from the reaction center chlorophyll $a$ P680 to pheophytin; pheophytin$^-$ to $Q_A^-$; and from $Q_A^-$ to $Q_B^-$. We shall end with a discussion of the role of $HCO_3^-$ in the reduction of plastoquinone.
PRIMARY REACTANTS AND REACTIONS: FEMTOSECONDS TO PICOSECONDS

Reactants.

P680. The existence of a reaction center Chl a in PSII, labeled as Chl a II, was first observed as an absorbance decrease at 690nm by Döring et al. (1967, 1969), and later by others (see reviews in Witt, 1975; Parson and Ke, 1982; Ke and Shuvalov, 1987). Floyd et al. (1971) measured these changes at 77K and labeled the responsible component as P680 by analogy with P700 of PSI. Since still earlier measurements in the 680-690nm region were shown to be artifacts of Chl a fluorescence (Rubinstein and Rabinowitch, 1963), it took many experiments and several years to accept this change to be due to an absorbance decrease (see e.g., Govindjee et al., 1970; Butler, 1972; Eckert et al., 1984). Finally, measurements of absorbance increase in the 820nm region (van Gorkom et al., 1975; Van Best and Mathis, 1978) established that the change was due to the oxidation of P680 to P680+, not due to Chl a fluorescence increase. Chl a cations (Chla+) show such a positive absorbance change (Fujita et al., 1978). Although the 820nm change is not as specific to P680+, it is easier to measure. It is now generally believed that: (a) P680 is a dimer in its ground state, but the positive charge in the cation (P680+) and the triplet (3P680) states are localized on a monomer (see e.g., Diner, 1986; Den Blanken et al., 1983); and (b) the redox potential (Em,7) of P680/P680+ is 1.1V (Jursinic and Govindjee, 1977; Klimov et al., 1980).

Recovery of P680 from P680+. The kinetics of the decay of P680+ to P680 are rather complex. Several components in the range of 200µs, 35µs, 5µs, 250 ns and 20-30 ns, have been observed. These may represent electron donation by electron donors and/or by reduced electron acceptors available for back reactions. The fastest (20-250 ns components representing 50-60% of the total signal) must be due to the main routes of electron flow from Z to P680+. These reactions have been measured by:

(1) Absorbance changes at 820nm (see e.g. van Best and Mathis, 1978, who measured a 30 ns component in dark-adapted thylakoids; Brettel et al., 1984; also see Schlödder et al., 1985) show that the kinetics of the reduction of P680+ depend upon the redox state of WOC; it is fastest when the WOC is in S_0 and S_1 states (20 ns), and slowest (50 and 260 ns; two phases of equal amplitude) in S_2 or S_3 state. This suggests that the coulombic force of the positive charge on the WOC slows the electron donation from Z to P680+. Furthermore, existence of two donors Z_1 and Z_2 was suggested. (For an earlier discussion of Z_1 and Z_2, see Govindjee and Jursinic, 1979.)
Chlorophyll a fluorescence increase. Mauzerall (1972) observed a Chl a fluorescence rise time of 20–30 ns in dark-adapted algae; Butler (1972) explained these data to be due to the disappearance of P680 \(^+\) which acts as a chlorophyll a quencher. Sonneveld et al. (1979), in an elegant analysis of Chl a fluorescence rise during a flash excitation at different light intensities, established that in dark-adapted thylakoids, this rise is best explained by a 20–40 ns reduction time of P680 \(^+\) to P680. However, in preilluminated samples, this rise was 400 ns. Whether these differences are due simply to different charged states of WOC, or, also due to different electron donors, (e.g. \(Z_1\) and \(Z_2\), see e.g. Bouges-Bocquet, 1980) is not yet known. Just as in kinetic data on absorbance changes, fluorescence changes also show microsecond components (see e.g. Jursinic and Govindjee, 1977).

**Pheophytin.** Pheophytin is a chlorophyll molecule in which magnesium in the center has been replaced by two hydrogens. Klimov et al. (1977) showed that Pheo\(^-\) can be accumulated in PSII if P680 \(^+\) is kept reduced by an external donor and \(Q_A\) is chemically reduced prior to illumination (see review by Klimov and Krasnovsky, 1981). Pheophytin a is different from chlorophyll a in several respects: (a) it absorbs more weakly in the red and more strongly at 505 and 535nm than Chl a (Fujita et al., 1980); (b) in vitro, it can be reduced more easily compared to Chl a (Fajer et al., 1980); (c) its midpoint redox potential is -0.6V, less negative than that of Chl a (Klimov et al., 1979; Rutherford et al., 1981); pheophytin exists in a monomer form (Klimov et al., 1980) in PSII, but there are 2 pheophytin molecules per reaction center (Murata et al., 1986; Omato et al., 1984). As suggested in Fig. 2, Pheo must sit quite close to P680 as no membrane potential seems to be observed.

**Reaction Center Preparation**

In order to measure the primary photochemical reaction, i.e., the charge separation, one needs to have a purified, stable reaction center preparation that is free of antenna Chl a molecules. Nanba and Satoh (1987) isolated a complex that contains only three proteins: D\(_1\), D\(_2\) and cytochrome b-559 (also see Satoh et al., 1987; Barber et al., 1987; Márder et al., 1988; Seibert et al., 1988). This preparation contains only 4 or 5 Chl a, 2 Pheo, and a single \(\beta\)-carotene. Since Pheo a could be reduced by illumination, it was suggested that this complex is analogous to the reaction center from photosynthetic bacteria [see e.g. that the latter contains 4 Bchl and 2 B Pheo molecules (Deisenhofer et al., 1984)]. Unfortunately, the Nanba-Satoh preparation lacks the bound quinones \(Q_A\) and \(Q_B\), and has not been crystalized for structural and functional
studies. However, herbicide binding sites are shown to exist (Giardi et al., 1988). Akabori et al. (1988) have, however, prepared fractions that contain the bound quinones but their method is much more involved. Marder et al. (1987) have demonstrated that D-1 and D-2, in their preparation, are indeed the products of appropriate genes; and Chapman et al. (1988) have measured electron flow properties of these preparations. Although electron flow from Pheo$^-\text{A}$ cannot be measured, these preparations are highly suitable for measurements on primary charge separation. We shall review below our results, obtained in collaboration with Doug Johnson (of Argonne) and Mike Seibert (of SERI) (Wasielewski et al., 1989). A major reason for our success was the availability of a preparation that is stable and remains optically clear for an entire day at 4°C under intense illumination ($10^5$ laser pulses). The stability and clarity were obtained (H. McTavish, R. Picorel and M. Seibert, 1988) through the following: (1) reaction center was concentrated with polyethylene glycol (a stabilizer); (2) addition of low concentration (final 0.04%) of Triton X-100 keeps the material from aggregating; (3) the material was kept under anaerobic conditions by the addition of glucose, catalase, and glucose oxidase.

**Photochemistry**

The primary reactions can be written as:

$$^1\text{Chla} + h\nu \rightarrow ^1\text{Chla}^*$$ (light absorption; creation of excited state)

$$^1\text{Chla}^* + \text{P680} \rightarrow ^1\text{Chla} + ^1\text{P680}^*$$ (creation of singlet excited state of P680)

$$^1\text{P680}^* + \text{Pheo} \rightarrow ^1\text{P680}^+ + \text{Pheo}^-$$ (primary charge separation)

$$^1\text{P680}^+ + \text{Pheo}^- \rightarrow \text{P680} + \text{Pheo}$$ (charge recombination, or loss of charges by other mechanisms)

$$^1\text{P680}^+ + \text{Pheo}^- \rightarrow ^3\text{P680} + \text{Pheo}$$ (triplet state formation)

The act of light absorption is the most rapid reaction and is estimated from the interaction of light with Chl $a$ molecule. For example, Chl $a$ goes into first singlet excited state by absorbing red light (680nm). The 680nm light oscillates with a frequency of $4.41 \times 10^{14}$ cycles s$^{-1}$. Thus,
one transition (or cycle) occurs in 2.5 \times 10^{-15}s or 2.5 femtoseconds. This, then, is the approximate time of excitation (reaction 1). The excitation energy transfer in a reaction center must also be very rapid, considering the rise time for \(^1\text{P}_{680}^*\) production to be within the 500 fs instrument function (reaction 2). Wasielewski et al. (1989) have measured the lifetime of reaction 3 to be 3.0 ± 0.6 picoseconds in stable reaction center II preparations (to be discussed in details). The lifetime of P680\(^+\) - Pheo\(^-\) (reaction 4) had been measured in Nanba and Satoh preparations to be 32 nanoseconds (ns) by Takahashi et al. (1987) and Danielius et al. (1987). Hansson et al. (1988) have reported that the lifetime of P680\(^+\) - Pheo\(^-\) depends upon the number of antenna Chl \(a\) remaining attached to the reaction center. Finally Okamura et al. (1986) have shown the formation of the triplet state of P680, and have established the radical pair nature of its precursor through its spin polarization characteristics. Nuijs et al. (1986a,b) have studied primary reactions, using a 35ps laser, in photosystem II particles enriched in reaction centers (P680/80 Chl). This study, however, does not provide direct answers to the steps involved in reaction 3 above. We discuss below the newest finding, that is, the first direct measurement of the kinetics of charge separation in a stable reaction center of plant photosynthesis, using 500 fs laser flashes.

**Charge Separation in Photosystem II Reaction Center**

Wasielewski et al. (1989) have provided the first direct measurement of charge separation in stabilized PSII reaction centers with a 500 fs resolution. Using 610nm exciting flashes, they observed the following: (1) an instrument, limited (0.5 ± 0.4 ps) decrease in absorbance at 670nm and 485nm indicating the formation of the singlet excited state of P680 \((^1\text{P}_{680})\); (2) an increase in absorbance at 820nm (due to the formation of P680\(^+\)) with a time constant (1/e) of 3.0 ± 0.6 ps (Table I); (3) a decrease in absorbance at 670nm and an increase in absorbance at 485nm or at 538nm reflecting the formation of P680\(^+\) Pheophytin\(^-\) with a time constant also of approximately 3 to 4 ps; and (4) a decrease in absorbance at 650nm (at the isosbestic point for the P680\(^+\) Pheo\(^-\) - P680 Pheo changes), reflecting the decay of \(^1\text{P}_{680}\) with a time constant of 2.6 ± 0.6 ps that matches, within experimental error, the formation of P680\(^+\) (Fig. 5). Furthermore, it was shown that when Pheo was kept reduced by the addition of sodium dithionite, methylviologen and light, only the absorbance changes due to the formation of \(^1\text{P}_{680}\) were observed; the absorbance increase at 820nm was eliminated confirming that the observed changes are indeed due to the charge separation.

The above conclusion was further supported by quantitative comparisons of the absorbance changes measured at various wavelengths (Table II). For example, the expected ratio of absorbance at 800nm (due
### Table I. Summary of Rise and Decay Components (From data of Wasielewski et al., 1989)

<table>
<thead>
<tr>
<th>λ, nm</th>
<th>absorbance</th>
<th>time</th>
<th>components</th>
</tr>
</thead>
<tbody>
<tr>
<td>820</td>
<td>increase</td>
<td>3.0 ± 0.6ps</td>
<td>P680(^+) formation</td>
</tr>
<tr>
<td>670</td>
<td>decrease</td>
<td>0.5 ± 0.4ps</td>
<td>Instrument-limited, P680(^*) formation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.3 ± 0.4ps</td>
<td>P680(^+) and Pheo(^-) formation</td>
</tr>
<tr>
<td>650</td>
<td>decrease</td>
<td>2.6 ± 0.6ps</td>
<td>Decay of P680(^*) (isosbestic for both P680(^+) and Pheo(^-))</td>
</tr>
<tr>
<td>538</td>
<td>increase</td>
<td>2.8 ± 0.6ps</td>
<td>P680(^+) and Pheo(^-) formation</td>
</tr>
<tr>
<td>485</td>
<td>decrease</td>
<td>0.5 ± 0.4ps</td>
<td>Instrument-limited, P680(^*) formation</td>
</tr>
<tr>
<td></td>
<td>increase</td>
<td>3.6 ± 0.8ps</td>
<td>P680(^+) and Pheo(^-) formation</td>
</tr>
</tbody>
</table>

to P680\(^+\)Pheo\(^-\)) and that at 538nm trough (due to Pheo\(^-\)) of 5 (2 x 10\(^4\) M\(^-1\) cm\(^-1\)/4 x 10\(^3\) M\(^-1\) cm\(^-1\)) was matched by the same observed ratio (ΔOD at 800nm = 0.035/ΔOD at 538nm trough = 0.007). The same conclusion was obtained from the ratio of absorbance changes at 674nm to that at 800nm, where the extinction coefficient ratio was 4.0 (8 x 10\(^4\) M\(^-1\) cm\(^-1\) at 674nm/ 2 x 10\(^4\) M\(^-1\) cm\(^-1\) at 800nm) and the measured absorbance ratio was 4.3 (OD 674nm = 0.15/OD at 800nm = 0.035). Wasielewski et al. (1989) further concluded that: (1) no other intermediate exists between P680 and pheophytin at times > 500 fs, and (2) the quantum yield of charge separation is about 1.0.

### ELECTRON FLOW FROM PHEOPHYTIN TO Q\(_A\): PICOSECONDS TO A NANOSECOND

Since the back reaction between Pheo\(^-\) and P680\(^+\) has been measured to be 30ns (Takahashi et al., 1987; Danielius et al., 1987) in Satoh and Nanba's D-1, D-2, cyt b-559 preparations, and is expected to be 2-4ns in intact PSII, the forward electron flow from Pheo\(^-\) to Q\(_A\) is expected to be many orders of magnitude faster. Based on the measurements of the lifetime of chlorophyll \(a\) fluorescence of 180 to 300 ps from PSII in algae
Figure 5. Transient absorption changes at 820 nm (due to formation of P680\(^+\)) and at 650 nm (due to decay of \(^1\)Chla\(^+\)) for photosystem II reaction centers following a 100 \(\mu\)J, 500 fs laser flash at 610 nm. Left: 650 nm change; right: 820 nm change. (After Wasielewski et al., 1989.)

when the reaction centers are fully open, the reduction time of \(Q_A\) is suggested to be <300 ps (see Moya et al., 1986; Holzwarth, 1987, for reviews on lifetime of fluorescence measurements). Our own (J. Fenton, N. S. Rao, E. Gratton, 1982, presented at the Midwest Photosynthesis Congress, Argonne, IL, USA) unpublished measurements of 200 ps in spinach thylakoids is in agreement with this conclusion. Since P680\(^+\) to P680 reaction occurs in 20–30 ns (P680\(^+\) is a quencher of Chl \(a\) fluorescence), the reduction time of \(Q_A\) to \(Q_A^-\) cannot be measured by fluorescence. However, the electron flow from Pheo\(^-\) to \(Q_A\) can be measured by absorbance changes. Nuijs et al. (1986a), from measurements with 35 ps resolution, concluded that the electron transfer from Pheo\(^-\) to \(Q_A\) occurs with a time constant of 270 ps in spinach PSII preparations enriched in P680 (\(^1\)P680/80 Chl \(a\)). On the other hand, Schatz et al. (1987), using Synechococcus PSII preparations enriched in P680 (also \(^1\)P680/80 Chl \(a\)), estimated that the electron transfer from Pheo\(^-\) to a subsequent quinone electron acceptor takes place in \(\sim 500\) ps. Furthermore, the quantum yield of photoreduction of \(Q_A\) has been estimated to be 0.9 (see Thielem and van Gorkom, 1981).

Since electron flow from Pheo\(^-\) to \(Q_A\) is assumed to traverse a large portion of the thylakoid membrane, a membrane potential (\(\Delta\Psi\)) is expected to be produced. Measurements of Trissl and Kunze (1985) by macroelectrodes suggest that \(\Delta\Psi\) in PSII occurs in the hundreds of picosecond range, and the flash-induced reduction of pheophytin is not
Table II. Magnitudes of Absorbance Changes (From Data of Wasielewski et al., 1989)

<table>
<thead>
<tr>
<th>λ, nm</th>
<th>Component</th>
<th>Extinction coefficient M⁻¹ cm⁻¹</th>
<th>ΔOD, observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>800</td>
<td>P680⁺</td>
<td>(10^4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pheo⁻</td>
<td>(10^4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P680⁺ + Pheo⁻</td>
<td>(2 \times 10^4)</td>
<td>0.035</td>
</tr>
<tr>
<td>538</td>
<td>Pheo⁻</td>
<td>(4 \times 10^3)</td>
<td>0.007</td>
</tr>
<tr>
<td>Ratio 800/538</td>
<td>--</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>674</td>
<td>P680⁺ + Pheo⁻</td>
<td>(8 \times 10^4)</td>
<td>0.15</td>
</tr>
<tr>
<td>Ratio 674/800</td>
<td>--</td>
<td>4</td>
<td>4.3</td>
</tr>
</tbody>
</table>

electrogenic. This confirms the picture that P680 and pheophytin are on the same side of the membrane and \(Q_A\) is on the other side.

**ELECTRON FLOW OUT OF \(Q_A\): THE \(Q_A\) TO \(Q_B\) REACTION: NANOSECONDS TO A MILLISECOND**

**Reactants.**

\(Q_A\): The major electron acceptor \(Q_A\) is a plastoquinone permanently bound on the \(D_2\) protein. There is a tremendous heterogeneity among the quinone acceptors in PSII (see e.g. Vermaas and Govindjee, 1981; Black et al., 1985; Diner, 1986; Rich and Moss, 1987). Since it is the second acceptor of PSII, the first being pheophytin, it is also referred to as \(AII_2\). Originally, it was called "Q" for quencher of fluorescence (Duyssens and Sweers, 1963; see a still earlier suggestion by Kautsky et al., 1960), and is equivalent to \(Q_1\) of P. Joliot, and, most likely, has a \(E_m\) of 0 mV (see e.g. Horton and Croze, 1979; Malkin, 1982). Its operating potential could be as low as -130mV. It is assigned to be equivalent to \(Q_H\) (a Q high potential). (For an earlier discussion of \(Q_1\) and \(Q_2\), see R. Govindjee et al., 1970.)
When $Q_A^-$ is reduced to the semiquinone $Q_A^-$, a characteristic absorbance change at 320nm is observed, and is labeled as X-320 (discovered by Stiehl and Witt (1968). (See Schatz and van Gorkom, 1985, for a recent UV spectra.) Although the protein (or a group) near $Q_A$ may become protonated (Rutherford and Zimmerman, 1984), no evidence of protonation of $Q_A^-$ is observed even at pH4.0 (van Gorkom, 1974).

$Q_A^-$ interacts with pheophytin$^-$ as well as a non-heme iron (see e.g. Klimov et al., 1980). Although there is no apparent reason for believing that the antenna complexes CP-43 and CP-47 are involved in any chemistry, deVitry et al. (1984) have observed that both these complexes were necessary for $Q_A$ reduction.

All the permanently bound acceptors in the so-called PSI$\alpha\alpha$ are also referred to as Q$\alpha$ and those in PSI$\beta\beta$ as Q$\beta$. There is the possibility that the low potential Q, $Q_L^-$, may be equivalent to $Q_2$ (Joliot and Joliot, 1981), or Xa (Eckert and Renger, 1980). Neither Q$\gamma$ nor Xa is a quinone. The so-called $Q_{400}$ that has been shown to be Fe (see Pterouleas and Diner, 1986) of the $Q_A^-$Fe$^{3+}$Q$B$ complex cannot be equivalent to $Q_L^-$. No membrane potential ($\Delta\Psi$; C-550) is associated with $Q_2^-$ or $X_a^-$, but $Q_A^-$ production shows $\Delta\Psi$, and, an associated C-550 change that involves pheophytin and carotene.

In view of the above, it is not clear whether there exists only one or two tightly bound iron-quinones per reaction center in PSI. Brettel et al. (1985) find evidence for only one, but Evans and Ford (1986) claim that there are two tightly-bound iron quinones. Relationship to the PSI$\alpha\alpha$ and PSI$\beta\beta$ centers must be examined before we can reach any conclusions.

$Q_B$. There has not been much discussion of heterogeneity of $Q_B$ except that there are two types of centers: "B type" (i.e., those that go through a two electron gate, see explanation below) and those that are "non-B type" (i.e. those that do not go through a two electron gate) (see Lavergne, 1982). PSI$\beta\beta$ centers also do not go through the two electron gate (Thielen and van Gorkom, 1981). It is assumed that some non-B type centers exist in PSI$\alpha\alpha$ centers also. $Q_B$ is bound to the 32 kDa lysine-free protein; it is also a herbicide binding protein, as mentioned in the Introduction. We have suggested that it also binds two HCO$_3^-$ ions per polypeptide (see Blubaugh and Govindjee, 1988). Binding of the herbicide atrazine has been shown by photoaffinity labeling of $^{14}$C azido-atrazine (Gardner, 1981; Satoh et al., 1983). Herbicides (inhibitors) compete with $Q_B$ binding by binding on the apoprotein; they are suggested to displace $Q_B$ (Velthuys, 1981; Wraight, 1981). Herbicide resistance has been traced to alterations in single amino acids in the $Q_B$ apoprotein (see e.g. Erickson et al., 1985; Johanningmeier et al., 1987).
Oettmeier and Soll (1983) and Vermaas et al. (1984) have shown apparent competition of short-chain plastoquinone analogs with $^{14}$C-DCMU and $^{14}$C-atrazine, respectively. Lavergne (1982) has clearly shown that binding of DCMU to centers with $Q_B^-$ is much lower than with $Q_B^{2-}$; furthermore, Lavergne (1982) has also shown competition of $Q_B^-$ with benzoquinone. Recently, Wydrzynski and Inoue (1987) showed that artificial quinones can easily replace $Q_B$ and modify the properties of the PSI acceptor side.

It is of interest to us to mention that effects on the D-1 protein, usually on the acceptor side by mutation or by inhibitors, can show effects on the donor side of PSI (e.g. Golbeck and Warden, 1985 for salicylaldoxime; Metz et al., 1986 for herbicides; and Warden and Csorday, 1987 for linolenic acid).

**Electron Flow from $Q_A^-$ to $Q_B^-$**: Electrons are rapidly transferred out of $Q_A^-$ with a halftime of 200 to 600 $\mu$s (Forbush and Kok, 1968; Mauzerall, 1972; Zankel, 1973). Bowes and Crofts (1980) measured this time as a function of flash number and have obtained evidence that $Q_A^-Q_B \rightarrow Q_A^-Q_B^{2-}$ occurs in 200 to 400 $\mu$s and $Q_A^-Q_B \rightarrow Q_A^-Q_B^{2-}$ in 600–800 $\mu$s. The slowness of the second reaction may be due to electrostatic repulsion of the negative charge. In the presence of herbicides, that displace $Q_B^-$, electron flow out of $Q_A^-$ is blocked and takes seconds.

As hinted above, $Q_B^-$ is a two electron acceptor and acts as a two electron gate. Bouges-Bocquet (1973) and Velthuys and Amesz (1974), independently, reported evidence for the existence of what they have called "B" or "R", which they suggested accepts electrons from $Q^+Q_A^-$. Bouges-Bocquet (1973) measured the reduction of methylviologen (an electron acceptor for photosystem I only) in PSI light, and found that the number of electrons reaching methylviologen was higher after the 2 and 4 than after 1 and 3 preilluminating flashes in PSI; thus, it was suggested that a component B is reduced by the first PSI flash to $B^-$ and then to $B^{2-}$ by a second flash and only when $B^{2-}$ was formed were electrons transferred to the PQ pool, and then to PSI. Velthuys and Amesz (1974) first blocked the electron donor side of PSII, added artificial electron donors to donate electrons to $Z^+$ or P680$^+$, exposed the samples to a series of light flashes, and then measured DCMU-induced Chl $\alpha$ fluorescence increase (DF) by a weak measuring beam. They observed a binary oscillation in CF with peaks after odd number of flashes. This was explained as follows: after flash 1, $Q^-R^-$ is converted to $QR^-$; DCMU blocks further electron flow, equilibrium is shifted to $Q^+R^-$ and fluorescence is high as $Q^-$ is a condition for high fluorescence. After flash 2, $Q^-R^-$ is converted to $QR^{2-}$; electrons move out to the PQ pool, QR is produced; DCMU addition gives low fluorescence since the system
is in low fluorescent state as Q is a condition for low fluorescence. Thus, the concept of the "two electron gate" was born. "B" or "R" has now been renamed $Q_B^-$. The important concept as to how electrons are transferred from $Q_B^-$ to the PQ pool was suggested by Velthuys (1981): In this concept, after $Q_B^{2-}$ is formed, it exchanges with a PQ molecule on the $Q_B^-$ protein. The reactions can now be written as (see e.g. Stein et al., 1984):

Here, I = inhibitor, $K_s$ stands for equilibrium constants and PQ for plastoquinone.

The ratio of $Q_B^-:Q_B^{2-}$ in dark-adapted isolated thylakoids is approximately 7:3 (see e.g. Wollman, 1978). However, in intact chloroplasts (Govindjee, T. Ono, and Y. Inoue, unpublished, 1983), leaves (Rutherford et al., 1984), and in higher plant cells grown in tissue culture (Xu et al., 1988) it is 1:1. Figure 6 shows that the decay of Chl $a$ fluorescence, reflecting $Q_A^-$ to $Q_A$ decay, is identical after both flash 1 and 2 (Xu et al., 1988) suggesting 1:1 ratio for $Q_B^-:Q_B^{2-}$ in dark-adapted soybean cells.

**ROLE OF BICARBONATE IN PLASTOQUINONE REDUCTION**

Warburg and Krippahl (1958, 1960) (also see Franck, 1945) discovered that CO$_2$ (or HCO$_3^-$) stimulates electron flow in the Hill reaction. Warburg (see e.g., his paper in 1964) considered this phenomenon as evidence for CO$_2$ being the source of O$_2$ in photosynthesis. Although Metzner (1975) and Stemler (1982) have favored an important role of CO$_2$ (HCO$_3^-$) on the electron donor, i.e. the O$_2$ evolving side of PSII, we have
consistently obtained evidence for a major effect of CO$_2$ (HCO$_3^-$) on the electron acceptor, *i.e.*, the plastoquinone reduction side of PSII (see reviews by Govindjee and van Rensen, 1978; Vermaas and Govindjee, 1981, 1982; van Rensen and Snel, 1985; Govindjee and Eaton-Rye, 1986; Blubaugh and Govindjee, 1988). We believe that HCO$_3^-$ is required for the electron flow and the protons needed for plastoquinone reduction in PSII.

Our current picture is: CO$_2$ is the diffusing and HCO$_3^-$ is the active species (see *e.g.*, Sarojini and Govindjee$^a$, 1981; and Blubaugh and Govindjee, 1986). At the pH of the stroma, we expect enough (220μM) HCO$_3^-$, much higher than the estimated 35-60μM dissociation constant, to be present at ambient air levels. We consider that two tight HCO$_3^-$ binding sites exist on the Q$_B$-apoprotein, discussed above: (1) on the Fe of the Q$_A$-Fe-Q$_B$ complex, as suggested by Michel and Deisenhofer

$^a$Initially, the faster kinetics with CO$_2$ was misinterpreted for it being the active species, rather than the diffusing species.
A binding to Fe is supported by the experiments of Vermaas and Rutherford (1984), who observed a large formate-induced (i.e. $\text{HCO}_3^-$ removal) increase in the $Q_A^{-}\text{Fe}^{2+}$ complex EPR signal at $g = 1.82$. A role of $\text{HCO}_3^-$ in protonation, that may not involve the Fe site, was suggested first by experiments of Khanna et al. (1980) and later by Govindjee, J.J. van Rensen, J.F.H. Snel and W. Tonk (unpublished, 1983; see Govindjee and Eaton-Rye, 1986). The latter authors showed that a binary oscillation, superimposed on a period of 4 oscillation, of $H^+$ release to the lumen was abolished by $\text{HCO}_3^-$ depletion (confirmed, reversibility of the effect shown, and extended by van Rensen et al., 1988). These experiments may not provide any clue as to any direct effect of $\text{HCO}_3^-$ on the protonation reactions per se, since the dips at uneven flashes may not be due to protonation of $Q_B^{2-}$ but due to increased $H^+$ release during PQH$_2$ oxidation at even flashes. On the other hand, pH dependence of electron flow from $Q_A^{-}$ to $Q_B^{-}$, as measured by Chl a fluorescence decay, shows remarkable differences with and without $\text{HCO}_3^-$ present. In $\text{HCO}_3^-$-depleted samples, Eaton-Rye and Govindjee (1988) observed that at pH 7.5, two turnovers of the $Q_B$ "two-electron gate" were necessary before the maximum slowing down of $Q_A^{-}$ to $Q_B^{-}$ electron flow could be seen on subsequent flashes. By contrast, at pH 6.5, the slowed time was maximum only after one turnover of $Q_B^{-}$ (i.e., after two flashes). However, the maximum slowed time was much greater at pH 7.5 than at pH 6.5. After 1 or 2 flashes, however, the situation was reversed, i.e., the decay was greater at pH 6.5 than at pH 7.5 (Fig. 7). Although complicated, the phenomenon can be understood by considering that there are two effects of $\text{HCO}_3^-$ depletion: one, a conformational change and the other a protonation event. Whether the second is a result of the first or whether they are two separate effects remains to be determined. As a result of conformational change, binding of plastoquinone that forms $Q_B$ is suggested to be decreased leading to changes in rate of $Q_A^{-}Q_B^{-}\rightarrow Q_A^{-}Q_B^{-}$ reaction. The effect is pH dependent, being faster at alkaline pH. It is likely that this effect involves changes at the Fe site. However, after higher flash numbers, either the conformational effect exposes another protonation effect, or a new separate protonation effect becomes observable. At alkaline pH: absence of $\text{HCO}_3^-$ leads to a larger lack of $H^+$'s for the protonation of plastoquinone sites, thus, slowing down the $Q_A^{-}$ to $Q_B^{-}$ reactions. At
Figure 7. Plot of the times (t_{50}) at which \((Q_A^-)\) is 50% of maximum \([Q_A^-]\) in \(\text{HCO}_3^-\)-depleted (treated) membranes with measurements at pH 6.5 and pH 7.5. At pH 7.5, \((Q_A^-)\) decay is much slower than at pH 6.5 after four flashes suggesting the role of \(\text{HCO}_3^-\) in protonation reactions. The flash frequency was 1 Hz. (After Eaton-Rye and Govindjee, 1988.)

acidic pH, however, \(\text{HCO}_3^-\) has a lesser role to play due to the greater availability of protons. This explains the larger slowing down at pH 7.5 than at pH 6.5.

The concept of two binding sites was further supported by the data of Blubaugh and Govindjee (1988b) who showed by kinetic analysis that the residual activity in \(\text{HCO}_3^-\)-depleted thylakoids was due to endogenously bound \(\text{HCO}_3^-\); the amount of Hill activity restored to \(\text{HCO}_3^-\)-depleted thylakoids by a half-saturating \([\text{HCO}_3^-]\) was non-linear with respect to [Chl]. Thus, the essentiality of \(\text{HCO}_3^-\) was suggested. After corrections were made for endogenous \([\text{HCO}_3^-]\), the activity versus \([\text{HCO}_3^-]\) no longer followed Michaelis-Menten kinetics, and suggested the existence of two cooperative binding sites (\(n > 1.4\)).

A detailed model of how \(\text{HCO}_3^-\) may function as a \(\text{H}^+\) donor was discussed by Blubaugh and Govindjee (1988) (see Fig. 8). It is proposed that when \(Q_B^-\) (PQ) is reduced to \(Q_B^-\), the pKa of a nearby histidine shifts
Figure 8. A working model for the involvement of $\text{HCO}_3^-$ in the protonation of $\text{Q}_B^-$. 1: Bicarbonate sits on an arginine (ARG), and shares its proton with a nearby histidine (HIS); PQ stands for $\text{Q}_B^-$. 2: $\text{HCO}_3^-$ replaces $\text{CO}_3^{2-}$, a $\text{H}^+$ has already been delivered to HIS. 3: Proton on HIS provides the positive charge needed to stabilize $\text{Q}_B^-$ formed after a light reaction. The $\text{CO}_3^{2-}$ is converted to $\text{HCO}_3^-$ by the uptake of a $\text{H}^+$ from the surrounding (see text for details). (After Blubaugh and Govindjee, 1988.)

from 6.4 to 7.9 (see Crofts et al., 1984) due to repulsive interaction between the negative charge on $\text{Q}_B^-$ and the lone pair of electrons on histidine. The pKa of the $\text{HCO}_3^-$ bound to a neighboring arginine is presumed to change from 10.2 to 7.9 due to electron withdrawal by
arginine. Thus, a $H^+$ is shared between $HCO_3^-$ and histidine. This $H^+$ ends up on histidine as another $HCO_3^-$ replaces the $CO_3^{2-}$ group holding the $H^+$ close to the histidine. This ensures irreversibility of $H^+$ transfer from $HCO_3^-$ to histidine. Since the pKa of free $CO_3^{2-}$ is 10.2, reprotonation is rapid. Finally, steric effects between the OH of $HCO_3^-$ and H on the histidine favors the transfer of $H^+$ from histidine to near $Q_B^-$ or $Q_B^{2-}$. We favor the possibility that arginine 257 and histidine 252 on D-1 protein may be the most likely pairs involved in protonation of $Q_B^-$ or $Q_B^{2-}$. Thus, they are our targets of site-directed mutagenesis in order to test the above hypothesis.

At present, this test can be done on a transformable cyanobacterium *Synechocystis* PCC 6803. It, thus, became necessary to check for the existence of bicarbonate effect in cyanobacteria. The only published work on this effect on cyanobacteria showed a negative result (van Rensen and Vermaas, 1981). However, results of J. Cao and Govindjee

![Figure 9](https://example.com/figure9.png)

**Figure 9.** Decay of $(Q_A^-)$, calculated from chlorophyll $a$ fluorescence decay, in thylakoids from *Synechocystis* PCC 6803. Open circles: Control; triangles: depleted of $HCO_3^-$ (25mM sodium formate); squares: restored with 5mM $HCO_3^-$ addition; solid circles: control with 5μM diuron. Note that $HCO_3^-$ depletion reversibly blocks the decay of $Q_A^-$ just as diuron does. (Data of J. Cao and Govindjee, 1988, unpublished.)
(unpublished, 1988) show the existence of HCO$_3^-$ effect in both thylakoids and intact cells of *Synechocystis* PCC 6803. Figure 9 shows that the decay of Q$_A^-$ after the third flash is dramatically slowed down upon bicarbonate depletion approaching the rate of decay in herbicide treated samples.

Photosynthetic bacteria, that have quite a different amino acid sequence on their electron acceptor side, do not show any significant bicarbonate effect (R. J. Shopes, D. Blubaugh, Govindjee and C. Wraight, unpublished, 1987). As noted earlier, Michel and Deisenhofer (1988) have suggested that in PSII, HCO$_3^-$ may replace the M-252 glutamate that provides the fifth ligand to the Fe. The possible relationship between the bacterial and plant systems as regards the second HCO$_3^-$ binding site (an arginine) has not yet been explored.

A major question about the HCO$_3^-$ effect is its role in vivo. A hint of this CO$_2$ effect was provided for wheat leaves by Gerbaud and André (1980). Govindjee, J. F. H. Snel and J. J. S. van Renssen (1983, unpublished; cited by Govindjee and Eaton-Rye, 1986) showed that bicarbonate depletion reversibly decreases the parabenzquinone Hill reaction by several fold and produces a Chl a fluorescence transient that mimics DCMU-treatment in *Scenedesmus* cells. Garab et al. (1983) showed similar changes in Chl a fluorescence transient in leaves when air or CO$_2$ was replaced with N$_2$. Mende and Wiessner (1985) showed that in *Chlamydomonas stellata*, but not in *Chlorella fusca*, replacement of air with CO$_2$-free-air first led to a decreased fluorescence suggestive of a blockage on the electron donor or water side, followed by an increase in fluorescence suggestive of a block on the electron acceptor side. Unfortunately, the parallel measurement on O$_2$ evolution was not done in identical conditions and perceived correlation between the two measurements was not established. Ireland et al. (1987), using wheat and maize leaves, showed that decreasing [CO$_2$] from already a low level (when CO$_2$ fixation was negligible) to a very low level caused changes that reflected a block between Q$_A^-$ and Q$_B^-$. Garab et al. (1988) showed that removal of CO$_2$ from the leaf atmosphere produced thermoluminescence bands indicating a preponderance of Q$_A^-$ over Q$_B^-$. Clearly showing that the absence of CO$_2$ blocked electron flow from Q$_A^-$ to Q$_B^-$. Ireland et al. (1987) working on wheat and maize, and Fatma El-Shintinawy and Govindjee (paper presented at the annual meeting of the American Society of Plant Physiology, 1988, Reno) working on spinach leaves showed that, when methylviologen is used as an electron acceptor (anaerobic conditions), absence of HCO$_3^-$ caused a faster fluorescence rise indicating a block in electron flow from Q$_A^-$ to Q$_B^-$. Addition of HCO$_3^-$ reversed the effect. Interestingly, El-Shintinawy and Govindjee observed that if the spinach leaves were infiltrated for a very short time (e.g., 10s) with 100 mM sodium formate, both O$_2$ evolution
and Chl a fluorescence levels decreased, apparently, suggesting a block on the electron donor side of PSII (Fig. 10). However, a longer infiltration (e.g., minutes) showed a dramatic decrease in O₂ evolution and a dramatic increase in the rate of Chl a fluorescence rise indicating a block on the electron acceptor side. It appears that the HCO₃⁻ pool associated with the first effect must be more loosely bound than that associated with the second effect. Our data on leaves, although reminiscent of Mende and Wiessner's data on Chlamydomonas, cannot be interpreted, however, as showing a block on the electron donor side because a block between pheophytin and QA (a site close to the Fe site) would also give the same result since reduced pheophytin is a quencher of Chl a fluorescence.

![Figure 10](image-url)

**Figure 10.** Parallel measurements on the rate of oxygen evolution (top curves) and chlorophyll a fluorescence changes in spinach leaf discs. (a): control; (b) infiltrated with 100mM sodium formate for 10s; (c): (b) restored with 1mM HCO₃⁻; (d): (b) restored with 10mM HCO₃⁻. Infiltration of leaf discs with 100mM sodium formate for longer time (> 1 min) shows elimination of O₂ evolution and a faster fluorescence rise to maximum fluorescence (data not shown). (After F. El-Shintinawy and Govindjee, 1988, presented at the 1988 Americal Society of Plant Physiology Meeting, Reno, Nevada.)

It appears to us that when HCO₃⁻ is completely removed, electron flow from QA⁻ to the plastoquinone pool is almost completely blocked after 1 or 2 turnovers of the two electron gate, (see Vermaas and Govindjee, 1982; Govindjee, 1984; Govindjee et al., 1984; J. Cao and Govindjee, unpublished, 1988). Experiments are in progress to further understand the mechanism of bicarbonate effect *in vivo.*
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REFERENCES


Blubaugh D, Govindjee (1986). Bicarbonate, not CO₂, is the species required for the stimulation of photosystem II electron transport. Biochim Biophys Acta 848:147-152.


Petroseas V, Diner BA (1986). Identification of $Q_{400}$, a high potential electron acceptor of photosystem II, with the iron of the quinone-iron acceptor complex. Biochim Biophys Acta 893:126-137.


