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12 Photosystem II Reaction Center and Bicarbonate

I. Introduction

All life on earth has been divided into three domains: archea, bacteria, and eukarya (Woese *et al.,* 1990). True photosynthesis involving redox components occurs only in the latter two. Photosynthetic bacteria, such as the well-known purple bacteria *Rhodobacter sphaeroides* and *Rhodopseudomonas*

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viridis, the green bacteria *Chlorobium thiosulfatophilum* and *Chloroflexus aurantiacus*, and the bacteriochlorophyll g-containing bacterium *Heliobacterium chlorum*, are all anoxygenic: they do not evolve oxygen. On the other hand, all cyanobacteria (e.g., *Synechocystis* sp.), algae (e.g., *Chlamydomonas reinhardtii*), and other photosynthesizing plants are oxygenic: they evolve oxygen.

A. Reaction center II: Structure and composition

Cyanobacteria and plants contain two types of reaction centers (RCs) (I and II) that operate in series to transfer electrons from water to $NADP^+$ (Govindjee and Govindjee, 1975; Andreasson and Vanngard, 1988). Purple photosynthetic bacteria and *Chloroflexus* type green bacteria contain RCs that are similar in several respects to RC II (Trebst, 1986; Barber, 1988; Michel and Deisenhofer, 1988; Rutherford, 1988), although a major difference is that they do not oxidize water and do not contain Mn.

A major breakthrough in photosynthesis research has been the crystallization and X-ray structure analysis of RCs of the photosynthetic bacteria *Rps. viridis* and *Rb. sphaeroides* (Deisenhofer and Michel, 1989; Feher *et al.*, 1989; Norris and Schiffer, 1990). Since the crystal structure of the RC photosystem II (PSII) is not yet available, the bacterial structure has been exploited to predict the structure of PSII. (For a diagram of the PSII RC, see Fig. 1.) The smallest complex capable of the primary charge separation on illumination appears to consist of the 33, 32, 10, and 4-kDa proteins (Satoh, 1988); these are the D2 and D1 proteins and the two polypeptides of cytochrome b_{559}. In addition, an increasing number of low-molecular-mass components is being found; a 5-kDa polypeptide labeled "I" has been established to be a component of the PSII RC (Ikeuchi *et al.*, 1989). Further, the RC of PSII contains 6 chlorophylls (Chls), 2 pheophytins (Phes), 2 plastoquinones (Q_A and Q_B), and a non-heme ion (see Kobayashi *et al.*, 1990, for Chls and Phes). Q_A sits in the D2 protein and is suggested, by analogy to photosynthetic bacteria, to remain bound to specific amino acids between helices IV and V. The non-heme iron sits in the middle, connected to four specific histidines on the D1 and D2 proteins. The location of Q_B is between helices IV and V of the D1 protein (Trebst, 1986). Table I lists the genes and gene products of PSII. For a review on the protein components of PSII, see Ikeuchi (1992).

B. Comparison with photosynthetic bacteria

1. Similarities

Since photosystem I (PSI) of green plant photosynthesis is anoxygenic, as is the photosystem of photosynthetic bacteria, it was thought for decades that these two photosystems should be comparable. In contrast, Trebst

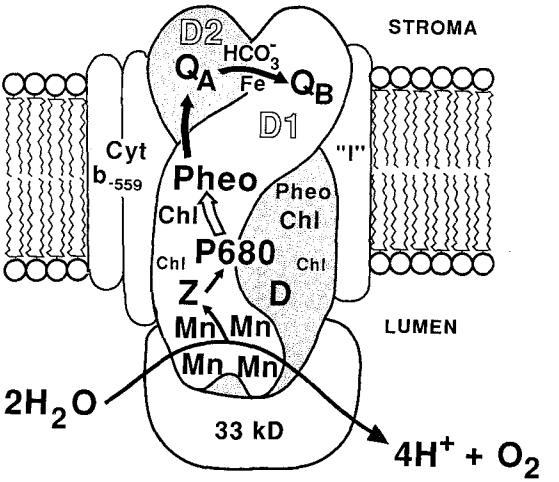

FIGURE 1 Schematic of the photosystem II (PSII) reaction center (RC) complex and the associated "nonessential" 33-kDa manganese stabilizing oxygen-evolving enhancing (OEE) complex. The RC complex is composed of D1, D2, cytochrome b_{559} and a *psbI* gene product, a 4.5-kDa protein. The functions of cyt b_{559} and "I" remain unknown. The RC chlorophyll a P680 and the non-heme iron (Fe) are shared between D1 and D2. The tyrosine residue (Z; the electron donor to P680), the active pheophytin (Phe; the electron acceptor of PSII), and the secondary bound plastoquinone (Q_B) are located on D1. D2, however, harbors the primary bound plastoquinone (Q_A), the "inactive" Phe, and the slow donor (D; also a tyrosine). Although four Mn atoms are shown to be located on D1, interaction of Mn with D2 is implicit in current models. Four additional chlorophyll (Chl) molecules are suggested to be present on both D1 and D2, functioning in various roles: electron acceptor prior to active pheophytin, harvester of light energy or excitons, and possible electron donor under special conditions. Finally, one or two bicarbonate ions may be bound to D1/D2 to aid in the protonation of Q_B^- or to provide appropriate conformation to the RCII for its functioning on the electron acceptor side of PSII.

(1986) discussed that the RC of photosynthetic bacteria has many similarities with that of PSII. He compared the hydropathy index plots and the amino acid sequences of the L and M subunits of *Rps. capsulatus* with those of the D1 and D2 proteins of PSII. In these proteins, five hydrophobic segments were detected to be common to all four peptides. These segments are very hydrophobic and are long enough to span the 35 Å of the membrane as α-helices. The D1 protein is considered to be equivalent to the L and the D2 protein to the M subunit of the bacterial RC.

The bacterial and PSII RCs are also very similar in their chromophore contents. The bacterial RC contains 4 bacteriochlorophylls (Bchls), 2 bacteriopheophytins (Bphes), two ubiquinones Q_A and Q_B, and a non-heme iron (see Michel and Deisenhofer, 1988). As noted earlier, the PSII RC has 6 Chls,

TABLE I **Photosystem II Genes and Their Products**[a]

Gene	Location (eukaryotes)	Product	Approximate apparent molecular mass
*psb*A	chloroplast	D1	32,000
*psb*B	chloroplast	CP47	47,000
*psb*C	chloroplast	CP43	43,000
*psb*D	chloroplast	D2	32,000
*psb*E	chloroplast	cyt b_{559}	9000
*psb*F	chloroplast	cyt b_{559}	4000
*psb*H	chloroplast	10,000-M_r phosphoprotein	10,000
*psb*I	chloroplast	reaction center component	4800
*psb*J	chloroplast	?	—
*psb*K	chloroplast	PS II-K	2400
*psb*L	chloroplast	PS II-L	5000
*psb*M	chloroplast	PS II-M	4700
*psb*N	chloroplast	PS II-N	4700
*psb*O	nucleus	33,000-M_r protein (OEE1)	33,000
*psb*P	nucleus	24,000-M_r protein (OEE2)	24,000
*psb*Q	nucleus	18,000-M_r protein (OEE3)	18,000
*psb*R	nucleus	10,000-M_r protein	10,000
cab	nucleus (family)	several light-harvesting complexes	21,000 - 29,000

[a] Adapted from Vermaas and Ikeuchi (1991).

2 Phes, 2 plastoquinones Q_A and Q_B, and a non-heme iron (Kobayashi *et al.,* 1990).

In both cases, the primary photochemistry involves a rapid (3-ps) charge-separation step (Feher *et al.,* 1989; Wasielewski *et al.,* 1989). A remarkable common feature of PSII and the bacterial RC is the mechanism of reduction of quinone (Section I,C,1).

2. Differences

In spite of the similarities in the arrangement and function of PSII and photosynthetic bacterial RCs, significant differences exist between them. A major difference is that PSII oxidizes water to oxygen, whereas the photosynthetic bacterial system does not. In bacteria, a cyclic reaction produces a high-energy intermediate that is used to reduce NAD^+ by reversed electron flow using external hydrogen donors.

Differences also exist in the accessibility and the reactivity of Fe (Petrouleas and Diner, 1986; Zimmerman and Rutherford, 1986) and in sensitivity to various herbicides (Trebst, 1991; Oettmeier, 1992), although most herbicides act by displacing Q_B (Velthuys, 1981; Wraight, 1981). These differences have

been explained on the basis of the differences in the amino acid sequences and, thus, of the secondary and tertiary structures of the two systems.

A possible difference between PSII and bacterial RCs lies in the orientation of the primary donor. Van Mieghem *et al.* (1991) suggest that P680, the RC Chl of PSII, may be oriented 30° to the membrane, quite distinct from the orientation of the RC Bchl (Deisenhofer *et al.*, 1985).

It is important to this discussion that PSII of plants (Diner *et al.*, 1991; Govindjee, 1991; Van Rensen, 1993) and cyanobacteria (Cao and Govindjee, 1988; Nugent *et al.*, 1988), but not the RCs of photosynthetic bacteria (Shopes *et al.*, 1989; Govindjee *et al.*, 1991a; Wang *et al.*, 1992), show a bicarbonate-reversible inhibition of electron flow from Q_A^- to the quinone pool when the samples are treated with formate (Govindjee, 1991; Van Rensen, 1993) or nitric oxide (Diner and Petrouleas, 1990). This difference also must reflect differences in the architecture of D1-D2 and L-M subunits.

C. Function of photosystem II

PSII can be considered a water-plastoquinone oxidoreductase; using four photons, it transfers four electrons from two molecules of water to plastoquinone, producing molecular oxygen and two molecules of doubly reduced plastoquinone. The RC proper is described in this section, the electron acceptor side in Section I,C,1 and the donor side in Section I,C,2.

The primary electron donor of PSII, P680, appears to be a dimer (Van Kan *et al.*, 1990), but its triplet is localized on one of the Chls (see review by Renger, 1992). Although a role for a Bchla molecule preceding Bphe appears to have become more probable in photosynthetic bacteria (see, e.g., Bixon *et al.*, 1991), a similar role for Chla preceding Phe remains to be discovered. By analogy to photosynthetic bacteria (Holzapfel *et al.*, 1990), PSII primary photochemistry may occur as follows:

$$\text{P680 Chl Phe} + h\nu \xrightarrow{2\ \text{fs}} \text{P680}^* \text{ Chl Phe} \quad (1)$$

$$\text{P680}^* \text{ Chl Phe} \xrightarrow{3\ \text{ps}} \text{P680}^+ \text{ Chl}^- \text{ Phe} \quad (2)$$

$$\text{P680}^+ \text{ Chl}^- \text{ Phe} \xrightarrow{<1\ \text{ps}} \text{P680}^+ \text{ Chl Phe}^- \quad (3)$$

Wasielewski *et al.* (1989,1990) have observed that the time for charge separation, that is, formation of $P680^+ Phe^-$, is 3 ps at 4° C and 1.4 ps at 15 K. The primary reactants return to their original state after Phe^- transfers its electron to the primary quinone Q_A within 200 ps (Nuijs *et al.*, 1986; Eckert *et al.*, 1988) and $P680^+$ transfers its positive charge (hole) to the electron donor Z within 20-200 ns (Van Best and Mathis, 1978; Sonneveld *et al.*, 1979; Brettel *et al.*, 1984), depending on the flash number that determines the state(s) of the charge accumulator on the water oxidation side. Z has been

shown to be a tyrosine residue (Gerken *et al.*, 1988), Tyr 161, in the D1 protein (see, e.g., Debus *et al.*, 1988a; Metz *et al.*, 1989). However, Phe is thought to be hydrogen bonded to a glutamic acid, also on the D1 protein (Moenne-Loccoz *et al.*, 1989; Nabedryk *et al.*, 1990).

1. Electron acceptor side of PSII

A remarkable common feature of PSII and the bacterial RC is the mechanism of reduction of quinone. The reduced Bphe or Phe reduces a one-electron acceptor bound quinone Q_A; normally, Q_A^- does not accept another electron. The reduction of the secondary quinone Q_B to quinol (Q_BH_2) occurs according to the following scheme (see, for review, Crofts and Wraight, 1983):

$$Q_AQ_B + {}^1h\nu \rightarrow Q_A^-Q_B \quad (4)$$

$$Q_A^-Q_B \leftrightarrow Q_AQ_B^- \quad (5)$$

$$Q_AQ_B^- + H^+ \leftrightarrow Q_AQ_B^-(H^+) \quad (6)$$

$$Q_AQ_B^-(H^+) + {}^2h\nu \rightarrow Q_A^-Q_B^-(H^+) \quad (7)$$

$$Q_A^-Q_B^-(H^+) \leftrightarrow Q_AQ_B^{2-}(H^+) \quad (8)$$

$$Q_AQ_B^{2-}(H^+) + H^+ \leftrightarrow Q_AQ_B^{2-}(2\ H^+) \quad (9)$$

$$Q_AQ_B^{2-}(2\ H^+) \leftrightarrow Q_AQ_BH_2 \quad (10)$$

$$Q_AQ_BH_2 \leftrightarrow Q_A + Q_BH_2 \quad (11)$$

$$Q_A + \text{Quinone} \leftrightarrow Q_AQ_B \quad (12)$$

This scheme includes the concept of the two-electron gate (Bouges-Bocquet, 1973; Velthuys and Amesz, 1974): Q_B must be doubly reduced before the quinol, Q_BH_2, can be released from the reaction center. The semiquinone form, Q_B^-, binds very tightly to the D1 protein. The protonation involves initial binding of H^+ to an amino acid, rather than directly to Q_B^-. The involvement of Asp 213 on the L subunit of *Rb. sphaeroides* in protonation steps has been shown by site-directed mutagenesis studies by Takahashi and Wraight (1990). A role of an amino acid in protonation reactions in PSII has not yet been demonstrated.

2. Electron donor side of PSII

The major difference between anoxygenic bacterial RCs and PSII lies in the inability of the former to oxidize water to oxygen. P680/P680$^+$ has a very high redox potential (+1.2 V; see, e.g., Jursinic and Govindjee, 1977), whereas P870/P870$^+$ has too low a redox potential (+0.4 V) to oxidize water. In addition, the amino acid sequences of D1 and D2 on the lumen side of

PSII are such that they easily can bind Mn atoms needed for oxygen evolution (see, e.g., Coleman and Govindjee, 1987), whereas the L and M subunits bind cytochrome of the c type instead. The oxygen-evolving system of PSII is located on the lumen side of the thylakoid membrane and includes extrinsic proteins of 33, 23, and 17 kDa in higher plants. In cyanobacteria, 17- and 23-kDa polypeptides are missing (see, e.g., Stewart *et al.,* 1985). The absence of a 33-kDa polypeptide in a site-directed mutant of *Synechocystis* sp. PCC 6803 does not eliminate the process of O_2 evolution (see Burnap and Sherman, 1991; Mayes *et al.,* 1991; Philbrick *et al.,* 1991). For reviews on PSII and the oxygen-evolving complex, see Ghanotakis and Yocum (1990), Hansson and Wydrzynski (1990), Andersson and Styring (1991), and Vermaas and Ikeuchi (1991).

D. Bicarbonate effect

CO_2 is generally known to be required for photosynthesis. CO_2 is fixed by ribulose 1,5-bisphosphate carboxylase and further reduced to carbohydrate. However, CO_2 also appears to be involved in photosynthetic electron transport. Warburg and Krippahl (1958) discovered that CO_2 accelerates the production of oxygen on illumination of isolated chloroplasts in the presence of an electron acceptor such as ferricyanide. This phenomenon was confirmed by many workers, but there was little agreement on the conditions necessary for observing the dependence of the Hill reaction on CO_2, or on the significance of such dependence. Stemler and Govindjee (1973) described a method by which reproducible and large increases in the rate of the Hill reaction in isolated thylakoids could be observed on addition of bicarbonate to samples treated in a specific manner. The method depends on depletion of CO_2 from thylakoids by flushing acetate- or formate-containing suspensions (pH 5.6–6) in the dark with CO_2-free air or pure nitrogen gas. The resulting electron transport rate is then measured at pH 6.5 (usually). The electron transport rate in such samples is extremely low, but is restored to control values on the addition of bicarbonate (Fig. 2). In all probability, CO_2 is the diffusing species, whereas bicarbonate is the binding species. Since the stimulation is evoked by the addition of a bicarbonate solution to anion-inhibited CO_2-depleted thylakoids, the phenomenon usually is called the bicarbonate effect. Included in this phenomenon are the stimulatory effects of bicarbonate when the inhibitor is nitric oxide other weak acid anions (azide, nitrite, etc.), or even when no such inhibitors are used.

As mentioned earlier, this bicarbonate effect is absent in both purple and green photosynthetic bacteria, but is present in PSII of cyanobacteria and higher plants. Thus, it is clear from the outset that CO_2/HCO_3^- is *not* a requirement for quinol formation per se. However, it is bound *in vivo* in PSII and, when bound, apparently regulates the protonation events at the Q_B site

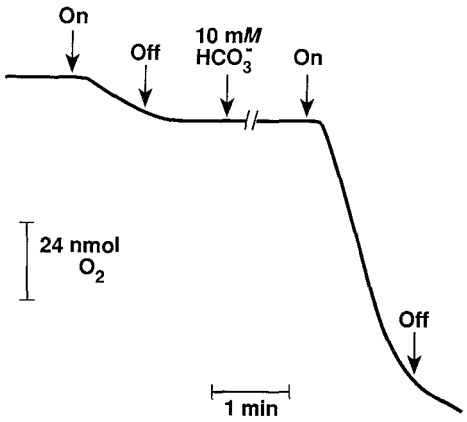

FIGURE 2 The bicarbonate effect on electron transfer in spinach thylakoids. The initial rate of electron transfer from H_2O to methylviologen was measured with and without bicarbonate in samples treated with 25 m*M* sodium formate under CO_2-free air. The rate of electron transfer in μeq/mg Chl/h without bicarbonate was 145; with bicarbonate, it was 1074 (pH 6.5). For details, see Eaton-Rye and Govindjee (1984).

and optimizes the formation of plastoquinol. Several reviews are available on this bicarbonate effect. Older literature can be found in Govindjee and Van Rensen (1978), and more recent information in reviews by Blubaugh and Govindjee (1988a), Govindjee (1991), and Van Rensen (1993).

II. Photosystem II electron acceptor side and the bicarbonate effect

Although Warburg (1964) had argued that CO_2 must be the source of oxygen in photosynthesis, contrary to the generally accepted idea that water is the source of oxygen, and although Stemler (1980) had advocated the idea that the bicarbonate effect was on water oxidation in PSII, all workers now agree that the major bicarbonate effect is located on the electron acceptor side of PSII. No bicarbonate effect is observed in PSI nor in photosynthetic bacterial RCs.

A. From Q_A to Q_B and the release of plastoquinol

The first indication for the bicarbonate effect to be on the electron acceptor side of PSII was obtained by Wydrzynski and Govindjee (1975), who measured Chla fluorescence induction kinetics in thylakoids in which artificial

electron donors replaced water and that had been treated specifically to obtain CO_2 depletion. The variable Chla fluorescence monitors the redox state of Q_A; Q_A is a quencher of fluorescence, whereas Q_A^- is not. Therefore, a rapid accumulation of Q_A^- due to an inhibition of electron transport beyond Q_A is detected easily by Chla fluorescence induction measurements. CO_2 depletion treatment in this system caused a fast increase in the variable fluorescence yield. This effect was reversed completely by bicarbonate readdition. The Chla fluorescence induction in the CO_2-depleted sample was similar but not identical to that observed in normal chloroplasts in the presence of the herbicide dichlorophenyldimethylurea (DCMU) (Wydrzynski and Govindjee, 1975). DCMU is known to block the reoxidation of Q_A^- by the secondary quinone acceptor Q_B by displacing it (Velthuys, 1981).

The decay of Chla fluorescence yield, in the μs to ms range, after a single-turnover brief and saturating flash reflects the reoxidation of Q_A^-. Under repetitive flashes, the half-time of the Q_A^- reoxidation was increased reversibly on CO_2-depletion treatment, indicating inhibition of Q_A^- reoxidation (Jursinic *et al.*, 1976). Detailed information about the bicarbonate effect on the reactions at the Q_AQ_B complex was obtained by measuring the decay of Chla fluorescence yield after various numbers of single-turnover brief and saturating flashes (see previous text). After even and uneven numbers of flashes, the following reactions (see Eqs. 4 - 12) occur:

$$Q_AQ_B \xrightarrow{^1h\nu} Q_A^-Q_B \leftrightarrow Q_AQ_B^- \overset{H^+}{\leftrightarrow} Q_AQ_B^-(H^+)$$

$$Q_AQ_B^-(H^+) \xrightarrow{^2h\nu} Q_A^-Q_B^-(H^+) \leftrightarrow Q_AQ_B^{2-}(H^+) \overset{H^+}{\leftrightarrow} Q_AQ_BH_2$$

$$Q_AQ_BH_2 \leftrightarrow Q_AQ_B$$

$$PQ \curvearrowright PQH_2$$

After protonation reactions are complete, Q_BH_2 is formed and exchanges with plastoquinone in the plastoquinone pool. Govindjee *et al.* (1976) found no differences in the decay of the Chla fluorescence yield after various numbers of flashes (1 through 10) in control and in anion-treated CO_2-depleted thylakoids to which bicarbonate was added. In CO_2-depleted anion-treated thylakoids, however, a very prominent slowing of the fluorescence decay was observed after three or more flashes, but not after the first or the second flash. Robinson *et al.* (1984) showed that, in thylakoids extensively treated under CO_2-depletion conditions, the Q_A^- decay, even after the first flash, is about 5-fold slower than in control thylakoids, but after 3 or more flashes the decay is approximately 40-fold slower. These experiments showed a much smaller inhibition of CO_2-depletion treatment on Q_A^- reoxidation by Q_B, but a very large inhibition after $Q_A^-Q_B^{2-}$ had been formed. Such results suggest a bicarbonate-reversible block in protonation reactions or the release of Q_BH_2.

Early suggestions were made that formate may interfere with protonation reactions near Q_B (Govindjee and Van Rensen, 1978). H_2CO_3 is possibly involved in the protonation of Q_B^-, Q_B^{2-}, or its proteinaceous environment, since the pK_a of (CO_2 + H_2O) is 6.4 at 25° C. $CO_2/HCO_3^-/CO_3^{2-}$ could, in principle, serve as a proton shuttle between Q_B and the external aqueous phase. Formate may not be able to function in such a manner, because its pK_a is 3.8. Experimental support for the protonation idea has been provided by Govindjee and Eaton-Rye (1986) and by Van Rensen *et al.* (1988). Eaton-Rye and Govindjee (1988) and Xu *et al.* (1991) showed that CO_2 depletion by anion treatment leads to a significant bicarbonate-reversible slowing of reoxidation of Q_A^- after the second and subsequent flashes, but not after the first flash (Fig. 3). These results suggest that bicarbonate may function in the protonation of Q_B^- needed for efficient formation of plastoquinol (see Eqs.

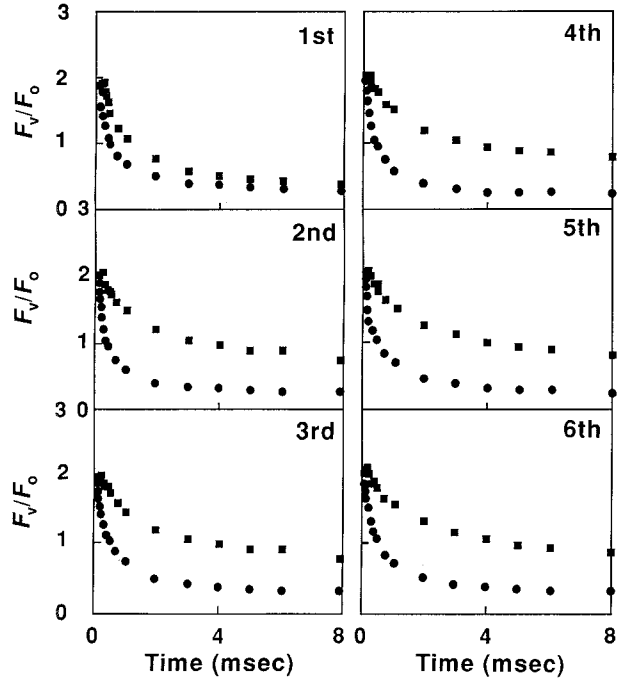

FIGURE 3 The bicarbonate effect on chlorophyll *a* fluorescence yield decay, monitoring the decay of [Q_A^-], after single-turnover μsec light flashes (1 through 6) at pH 6.5. Formate-treated/bicarbonate depleted, ■; 20 m*M* bicarbonate added, ●. Results with control spinach thylakoids were superimposed on those with bicarbonate. Note that formate treatment slowed [Q_A^-] decay more after the second and subsequent flashes than after the first flash. Further, bicarbonate fully reversed this effect. For details, see Xu *et al.* (1991).

4-10). Blubaugh and Govindjee (1988a) discussed a mechanism in which bicarbonate at specific arginine residues of the D1 protein protonates a specific histidine in the D1 protein as part of a mechanism for protonation of Q_B^-.

B. Non-heme iron

A kinetic analysis of the electron flow in different concentrations of thylakoids with and without a fixed subsaturating concentration of bicarbonate revealed a best fit that involves 1.4 cooperating binding sites (Blubaugh and Govindjee, 1988b). More than one binding site is suggestive of two binding sites or domains. Because the effect of bicarbonate is located on the electron acceptor side of PSII, the binding is expected to be close to the Q_A-Fe-Q_B complex of the D1-D2 proteins of the PSII RC. We may speculate that, of the two binding sites mentioned earlier, one binding site is at the non-heme iron and the other at an amino acid residue.

On the basis that only RCs of PSII, not those of photosynthetic bacteria, show the bicarbonate effect, Michel and Deisenhofer (1988) suggested that Glu 232 of the M subunit fulfills the role of bicarbonate in *Rps. viridis*. Since Glu M232 forms a ligand to iron, bicarbonate was suggested to form a bidentate ligand to iron in PSII. Several other experiments, for example, those using a chelator of iron, disulfuram (Blubaugh and Govindjee, 1988c), and those in which the redox potential of Fe was changed (Van Rensen *et al.*, 1988) were suggestive of the involvement of iron.

Vermaas and Rutherford (1984) showed that formate addition to thylakoids increases the amplitude of the $g = 1.82$ EPR signal of $Q_A^-Fe^{2+}$ tenfold (also see Nugent *et al.*, 1988). A formate/bicarbonate effect has been established clearly by EPR studies on the Q_A-Fe-Q_B complex (Bowden *et al.*, 1991). The Mossbauer spectrum of Fe is affected significantly by formate, and is returned to its original on readdition of bicarbonate, indicating that Fe is a key element in the binding of the formate that is displaced by bicarbonate (Diner and Petrouleas, 1987; Semin *et al.*, 1990).

NO has been shown to be able to ligate to the non-heme iron of PSII but not to that of *Rb. sphaeroides* RCs (Diner and Petrouleas, 1990). Associated with iron nitrosylation in PSII is the appearance of a $g = 4$, $S = 3/2$ EPR signal arising from the antiferromagnetically coupled Fe^{2+}-NO adduct. These observations imply that, in PSII, unlike in the bacterial RCs, the iron binds a ligand that is displaced easily by NO (Petrouleas and Diner, 1990). Kinetic measurements of Q_A^--to-Q_B electron transfer indicate that NO treatment produces the same pattern of slowing of quinone electron transfer as does formate (Diner and Petrouleas, 1990). Further, bicarbonate addition after treatment with NO leads to a suppression of the $g = 4$ Fe^{2+}-NO EPR signal and a restoration of normal electron-transfer kinetics. These observations imply

competition between NO and bicarbonate for ligation to the non-heme iron. Reversible binding of bicarbonate to the iron is consistent with its being a fully dissociable ligand. Diner *et al.* (1991) suggested two possible binding schemes: (1) formate binds to the iron at a site not occupied by NO but normally occupied by bicarbonate in the absence of NO or (2) formate binds outside the first coordinating sphere of the iron, competing, for example, with bicarbonate for binding to a cationic site or a hydrogen bond.

C. Comparison with purple and green photosynthetic bacteria

In spite of the known similarities of the D1 and D2 proteins of PSII and the L and M subunits of the purple photosynthetic bacterial RC, the latter do not show inhibition by formate or by nitric oxide that can be reversed by addition of bicarbonate. These differences must be due to differences in the amino acid sequence and, subsequently, in the architecture of the D1-D2 proteins versus the L and M subunits. The photosynthetic RC from the green bacterium *Cfl. aurantiacus* displays several differences from the purple photosynthetic bacteria: one of the monomer Bchl molecules is replaced by Bphe, Fe between Q_A and Q_B is replaced by Mn (see, e.g., Blankenship *et al.,* 1987), and several significant differences exist in their amino acid sequence (Ovchinikov *et al.,* 1988a,b). *Chloroflexus* RCs centers also appear to lack an H subunit.

Reaction centers from *Cfl. aurantiacus* showed, in contrast to PSII but similar to other photosynthetic bacteria (Shopes *et al.,* 1989), no significant difference between the control RCs and those treated with 100 m*M* formate (Govindjee, 1991; Govindjee *et al.,* 1991a). In addition, no difference was observed between the NO-treated (anaerobic) RCs and those treated with both NO and 10 m*M* bicarbonate. Thus, the differences between the amino acid sequence of the L and M subunits of *Cfl. aurantiacus* and those of purple photosynthetic bacteria appear not to be sufficient to bring about the binding of formate or NO.

To test the hypothesis of Michel and Deisenhofer (1988) that Glu M232 (Glu M234 in *Rb. sphaeroides*) is the equivalent of bicarbonate, Wang *et al.* (1992) replaced it with glutamine, glycine, and valine by site-directed mutagenesis and tested the mutants for electron flow from Q_A^- to Q_B and for the effects of formate. Since all mutants showed normal behavior of the electron acceptor complex in the presence or the absence of formate/bicarbonate, Glu M232 (234) appears not to be the equivalent of bicarbonate. Further, Glu M232 (234) may not be essential to the function of the electron acceptor complex.

In conclusion, the existence of bicarbonate-reversible formate and NO effects in PSII lies strictly in the unique amino acid sequence of the D1 and D2 proteins.

III. D1 protein and the bicarbonate effect

Studies on interactions of PSII herbicides and bicarbonate have provided important information about the site of the bicarbonate effect. More detailed knowledge was obtained from studies in which the thylakoid membrane was modified chemically, and from the use of herbicide-resistant mutants. All this knowledge has increased our insight into the function of the D1 protein of PSII. Figure 4 shows a model of the D1 protein.

A. Evidence for interactions of herbicides and bicarbonate

Interaction of diuron-type herbicides with plastoquinone was proposed first by Van Rensen (1969). Velthuys and Amesz (1974) suggested that diuron alters the midpoint potential of Q_A in such a way that it increases the difficulty with which Q_B can be reduced. The mechanism of herbicide action is now widely accepted to be a displacement of Q_B from its binding site (see Velthuys, 1981, for the PSII complex, and Wraight, 1981, for the RC of purple photosynthetic bacteria). The binding of herbicides appears to be competitive with plastoquinone; the rate of release of the inhibitor from the site is many times slower than the rate of release of plastoquinone (Vermaas *et al.*, 1984), implying that the inhibitor stays rather long at the binding site on the D1 protein in comparison with Q_B (Naber and Van Rensen, 1991). The inhibitory herbicides cannot be reduced by Q_A^- and electron transfer beyond this point is thereby prevented.

Interaction of herbicides with bicarbonate was shown by measurements of herbicide binding in the presence and absence of CO_2 and vice versa (Khanna *et al.*, 1981; Van Rensen and Vermaas, 1981; Vermaas *et al.*, 1982; Snel and Van Rensen, 1983). By adding different concentrations of bicarbonate to CO_2-depleted thylakoids, different rates of restoration of the Hill reaction were obtained. Plotting these reactions in thylakoids against bicarbonate concentration showed Michaelis-Menten kinetics that could be treated like an enzyme-versus-substrate system. From double reciprocal plots of the rate of the Hill reaction as a function of the bicarbonate concentration, the apparent dissociation constant (K_d) of the thylakoid-bicarbonate complex could be calculated. When 100 m*M* formate is present in the reaction medium, the apparent K_d appears to be about 1 m*M* bicarbonate. In the presence of low concentrations of formate, the apparent K_d decreases, approaching 80 μ*M* of total $NaHCO_3$ in the absence of formate (Snel and Van Rensen, 1984). When equilibrium concentrations of bicarbonate are used, this K_d drops to 40 μ*M* bicarbonate (Blubaugh and Govindjee, 1988a). The K_d for bicarbonate depends not only on the presence of formate, but also on the presence of herbicides. In the presence of urea, triazine, or phenol-type herbicides, the K_d for bicarbonate increases at least 2-fold, meaning that these

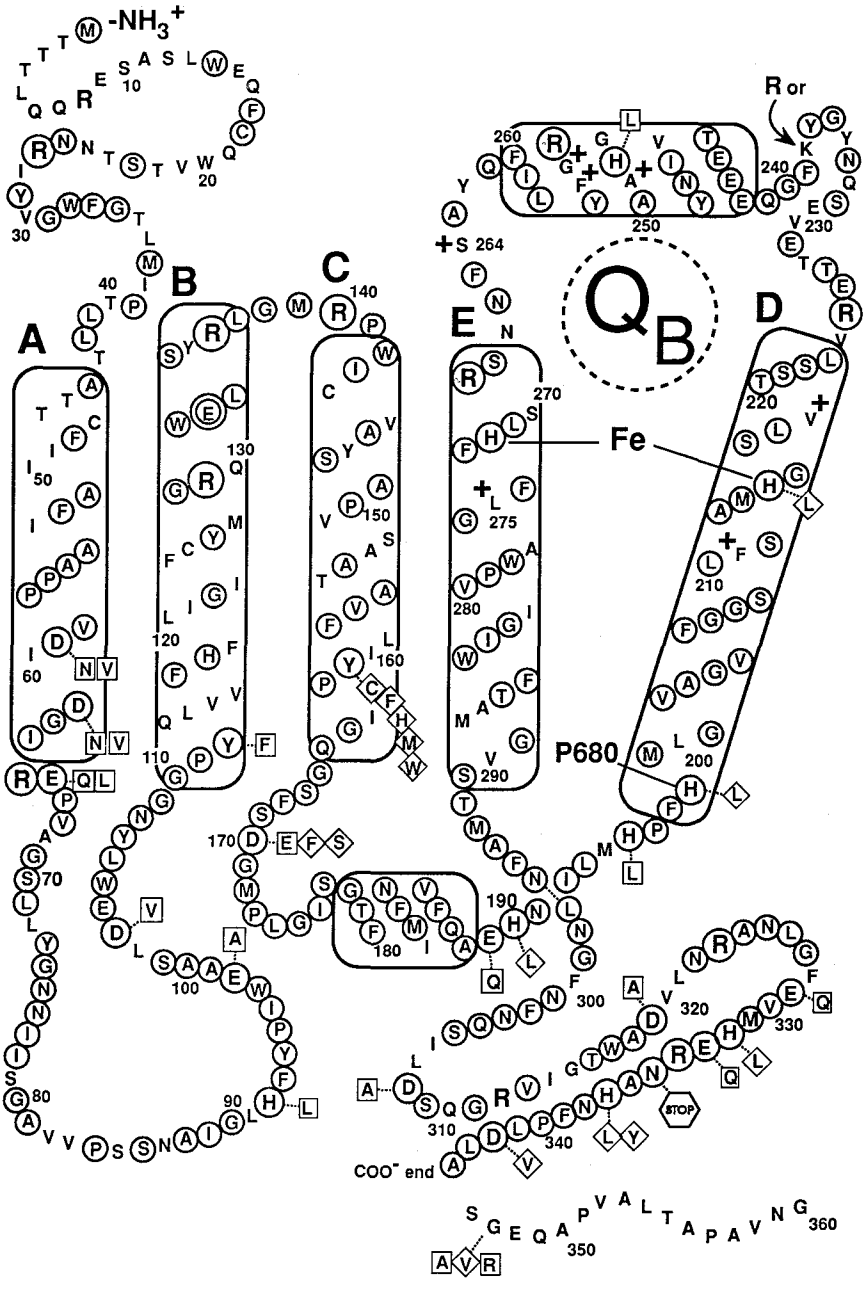

FIGURE 4 Schematic of a possible folding model of the D1 polypeptide of *Synechocystis* sp. PCC 6803, based essentially on the diagram by Nixon *et al.* (1992). Modifications include conserved amino acids (circled; see Andersson and Styring, 1991); locations of mutations leading to herbicide resistance; region where Q_B may be located. +, herbicide-resistant; □, PS^+; ◇, PS^-.

herbicides decrease the apparent affinity of the thylakoid membrane for bicarbonate. These arguments lead strongly to the suggestion that the binding site of bicarbonate is located on the D1 protein of PSII, since herbicides are known to bind to this protein (Oettmeier, 1992).

B. Chemical modifications

Trypsin-treated thylakoids lose the sensitivity of the Hill reaction to PSII herbicides; also, Q_A becomes accessible to ferricyanide (Van Rensen and Kramer, 1979). Such thylakoids show a lack of the bicarbonate effect in ferricyanide reduction (Khanna *et al.*, 1981; Van Rensen and Vermaas, 1981), indicating that the bicarbonate effect lies past Q_A and that the binding sites for bicarbonate and PSII herbicides may lie close to each other.

In thylakoids in which surface-exposed arginines or lysines were modified by treatment with phenylglyoxal or pyridoxal 5-phosphate, the rate of modification was faster in the presence than in the absence of bicarbonate. Vermaas *et al.* (1982) suggested that surface-exposed lysine or arginine residues are not involved in binding of bicarbonate to its binding protein, but that bicarbonate influences the conformation of its binding environment so the accessibility to amino acid modifiers is changed. These results could not answer questions regarding the role of arginines and lysines in the interior of the protein.

C. Use of herbicide-resistant mutants

The involvement of the D1 protein in the bicarbonate effect was first suggested from interactions with PSII herbicides. An additional indication was obtained using herbicide-resistant mutants. Khanna *et al.* (1981) observed an increased binding constant for bicarbonate in thylakoids of triazine-resistant *Amaranthus hybridus,* in which Ser 264 is changed to glycine in the D1 protein. In the presence of 100 m*M* formate, the binding constant of bicarbonate increases from 1 m*M* in the sensitive thylakoids to about 2 m*M* in the resistant ones, that is, the affinity for bicarbonate is lowered in the resistant membranes.

Using wild-type and herbicide-resistant mutants of the eukaryotic green alga *Chlamydomonas reinhardtii,* Govindjee *et al.* (1991b) demonstrated differential sensitivity of Chla fluorescence transients to 25 m*M* formate treatment. The most sensitive mutant was D1-S264A and the most resistant mutant was D1-L275F (see Fig. 4). The order of resistance (highest to lowest) was

$$\text{L275F} > \text{A251V} >> \text{wild-type} = \text{G256D} = \text{F255Y} = \text{V219I} >> \text{S264A}$$

These results show clearly the involvement of the D1 protein in the bicarbonate-reversible formate effect *in vivo.* This conclusion was confirmed

through studies on herbicide-resistant mutants of the cyanobacterium *Synechocystis* PCC 6714, with known amino acid changes in the D1 protein (Etienne *et al.*, 1990). Govindjee *et al.* (1990) measured oxygen yield in a sequence of flashes, Chla fluorescence transients, and Chla fluorescence yield decay (Q_A^- to Q_A reaction) after a single-turnover brief saturating flash. The resistance of cyanobacterial cells to formate treatment was in the following (highest to lowest) order:

double mutant D1-A251V/F211S > D1-F211S = wild-type > D1-S264A

(see Fig. 5). Bicarbonate addition restored all formate effects. Cao *et al.* (1992) showed that, in D1 mutants of *Synechococcus* sp. PCC 7942 in which D1-F255 was co-mutated with D1-S264, the hierarchy of the dissociation constant for bicarbonate (highest to lowest) was:

D1-F255L/S264A (46 μM) > D1-F255Y/S264A (31 μM)
~ D1-S264A (34 μM) ~ D1-F255Y (33 μM) > wild-type (25 μM)

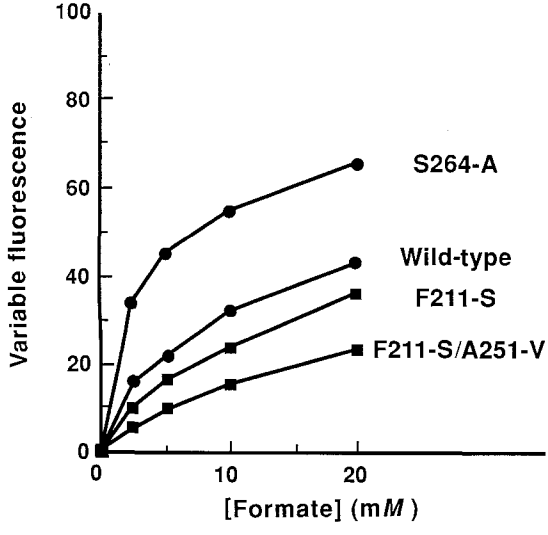

FIGURE 5 Chlorophyll *a* fluorescence as a function of formate concentration in cells of *Synechocystis* sp. PCC 6714 wild-type and mutants D1-S264A, D1-F211S, and D1-F211S/A251V. (See Fig. 4 for location of these mutations). Note the differential sensitivity of the mutants to bicarbonate-reversible formate effects. For details, see Govindjee *et al.* (1990).

These data suggest the importance not only of D1-S264, as before, but also of its environment in the bicarbonate-binding niche.

D. Function of the D1 protein

Helices D and E and the interhelical loop between them in both D1 and D2 proteins contain a rather high number of conserved amino acid residues (Svensson *et al.,* 1990). Therefore, it is quite logical that this part of the two proteins is most important to its function. The binding niche of the D1 protein for the physiological electron carrier Q_B and the PSII inhibiting herbicides is located between the D and E helices and the parallel DE helix, which is located in the stroma. This helix involves the amino acids 211 (Phe)–275 (Leu) of the D1 protein (Fig. 4). Our results suggest that formate and bicarbonate also bind in the same niche. To examine the involvement of specific amino acids in the D1 protein, studies of the bicarbonate effect in specific site-directed mutants must be made: D1-K(or R)235 and D1-R269 are strong contenders for such mutagenesis studies.

IV. D2 protein and the bicarbonate effect

On the basis of homologies with the RC of purple bacteria, the D2 protein, like D1, is thought to contribute to the binding of the reaction center P680 and the non-heme iron (Fig. 6). D2 is suggested to create most of the binding niche for Q_A and a Phe, whereas D1 harbors Q_B and another Phe (Trebst, 1986; Michel and Deisenhofer, 1988). Site-directed mutagenesis has led to the identification of the D1-Y161 residue as Z, the physiological electron donor to $P680^+$ (Debus *et al.,* 1988a; Metz *et al.,* 1989), and of the D2-Y160 residue of *Synechocystis* 6803 as the accessory slow PSII donor, D (Debus *et al.,* 1988b; Vermaas *et al.,* 1988). D2-H214 and D2-W253, which presumably interact closely with Q_A, are essential to the stability of the entire RC. Further, the E69 residue of D2 has been suggested to be a potential ligand to an Mn ion involved in photosynthetic oxygen evolution (Vermaas *et al.,* 1990). However, the role of the D2 protein in the bicarbonate effect was obscure until recently.

A. Use of site-directed mutants

A role for an arginine residue in bicarbonate binding was suggested first by Shipman (1981). The involvement of the D2 protein in the bicarbonate-reversible formate effect was established by the use of two site-directed *Synechocystis* sp. PCC 6803 mutants carrying mutations in two arginine resi-

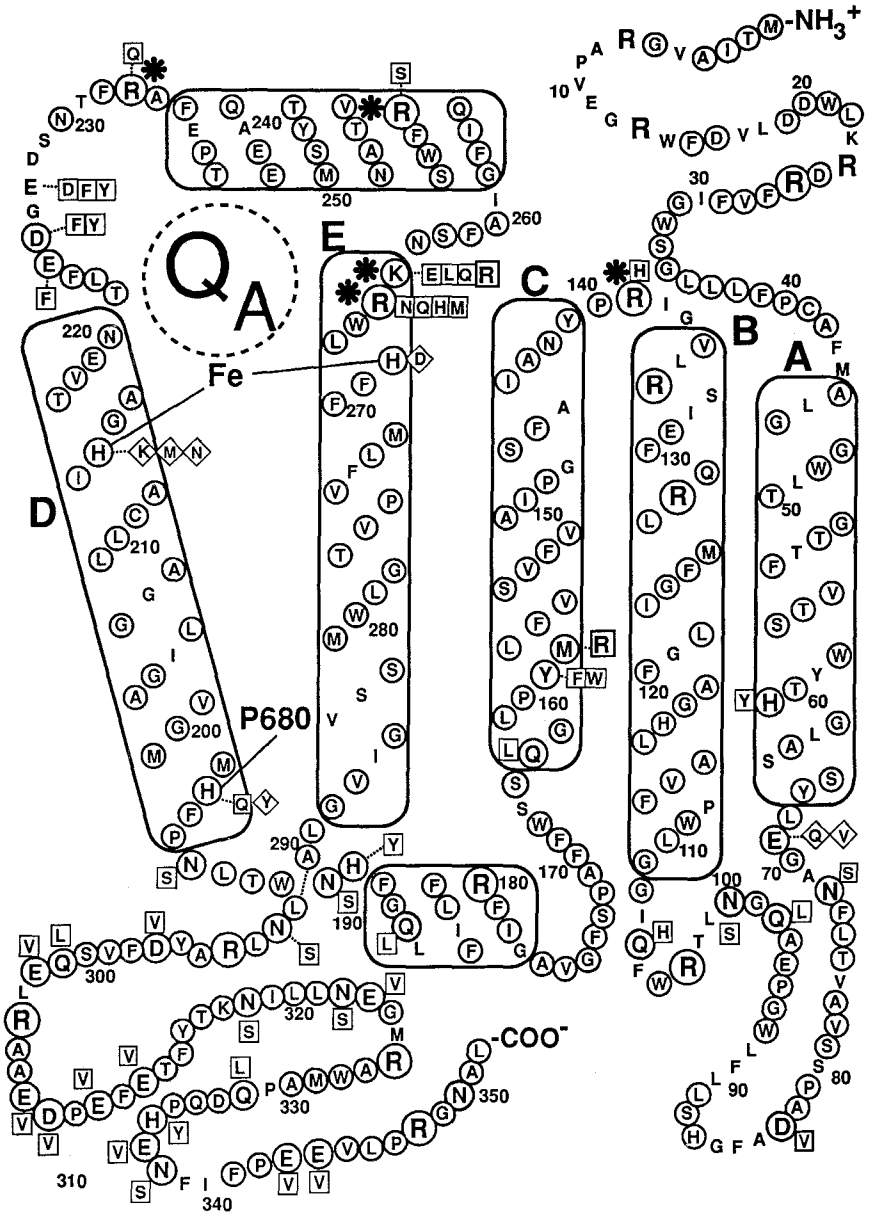

FIGURE 6 Schematic of a possible folding model of the D2 polypeptide of *Synechocystis* sp. PCC 6803, based essentially on the diagram by Nixon *et al.* (1992). Modifications include conserved amino acids (circled; see Andersson and Styring, 1991); locations of mutations affecting the bicarbonate-reversible formate effects; locations of some of the mutations studied by W. F. J. Vermaas and co-workers (see Pakrasi and Vermaas, 1992); region where Q_A may be located. □, PS^+; ◇, PS^-; *, bicarbonate effect.

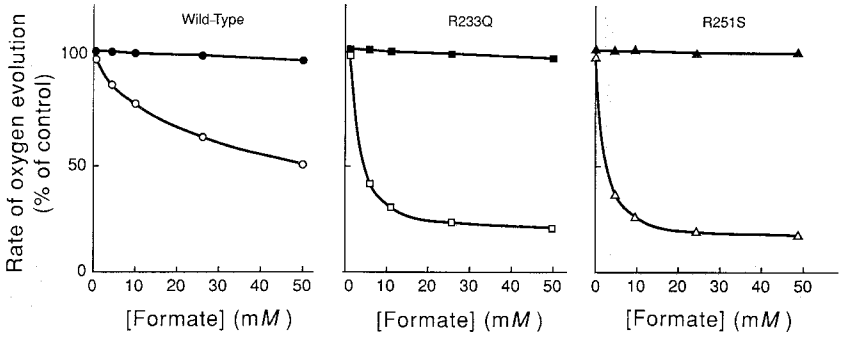

FIGURE 7 Steady-state oxygen evolution rate of *Synechocystis* sp. PCC 6803 cells of the wild-type and D2-R233Q and D2-R251S mutants at pH 6.8. (See Fig. 6 for location of these mutations.) The electron acceptor was a combination of dimethylquinone and ferricyanide. Note that the wild-type cells were inhibited 50% by ~50 m*M* formate, whereas the R233Q and R251S mutants by ~5 m*M* formate. For details, see Cao *et al.* (1991). However, the D2-R139H mutant behaved like the wild-type (Govindjee, J. Cao, S. Carpenter, and W. F. J. Vermaas, unpublished data), highlighting the specificity of R233 and R251 residues.

dues in the D2 protein (Cao *et al.*, 1991). The D2-R233Q and D2-R251S mutants are about 10 times more sensitive than the wild-type in the bicarbonate-reversible inhibitory effect of formate on the steady state rate of oxygen evolution (Fig. 7). This sensitivity was confirmed by measurements on Chla fluorescence decay and on oxygen evolution in flashing light. D2-R233 and D2-R251 were suggested to contribute to the stabilization of bicarbonate binding in PSII. The specificity of these arginines is confirmed when we observe that D2-R139H behaves exactly the way wild-type *Synechocystis* sp. PCC 6803 does in response to formate treatment (Govindjee, S. Carpenter, J. Cao, and W. F. J. Vermaas, unpublished results, 1990).

Diner *et al.* (1991) independently have constructed mutants in Lys 264 and Arg 265 in the D2 protein of *Synechocystis* 6803 (see Fig. 6). These D2-K264X and D2-R265X mutants had a slowed rate of electron flow from Q_A^- to Q_B and were very resistant to formate and NO treatment. Further, they showed a several-fold higher requirement for bicarbonate to increase the rate of electron flow from Q_A^- to Q_B, as though the binding sites for formate/NO/bicarbonate had been removed. These data suggest a significant role of D2-K264 and D2-R265 in the binding of CO_2/bicarbonate. The involvement of lysine opens up the question whether CO_2 also may be a binding species, forming a carbamate. This suggestion, of course, does not rule out a significant role of bicarbonate in either binding or function at another or at the same site.

B. Function of the D2 protein

The D2 protein, like the D1 protein, has five hydrophobic membrane-spanning helices of which the D and E helices appear to be the most important ones for its function on the electron acceptor side of PSII. The non-heme iron and Q_A are suggested to be bound here. The mutants D2-R233Q, D2-R251S, D2-K264X, and D2-R265X, discussed earlier, have alterations in the region between the D and E helices. This region may be in close contact with the comparable region in the D1 protein. Bicarbonate is likely to sit in the "cavity," making a bidentate ligand to Fe and H-bonds to neighboring amino acids (see Govindjee, 1991; Fig. 8, C. Gibas and Govindjee, unpublished results).

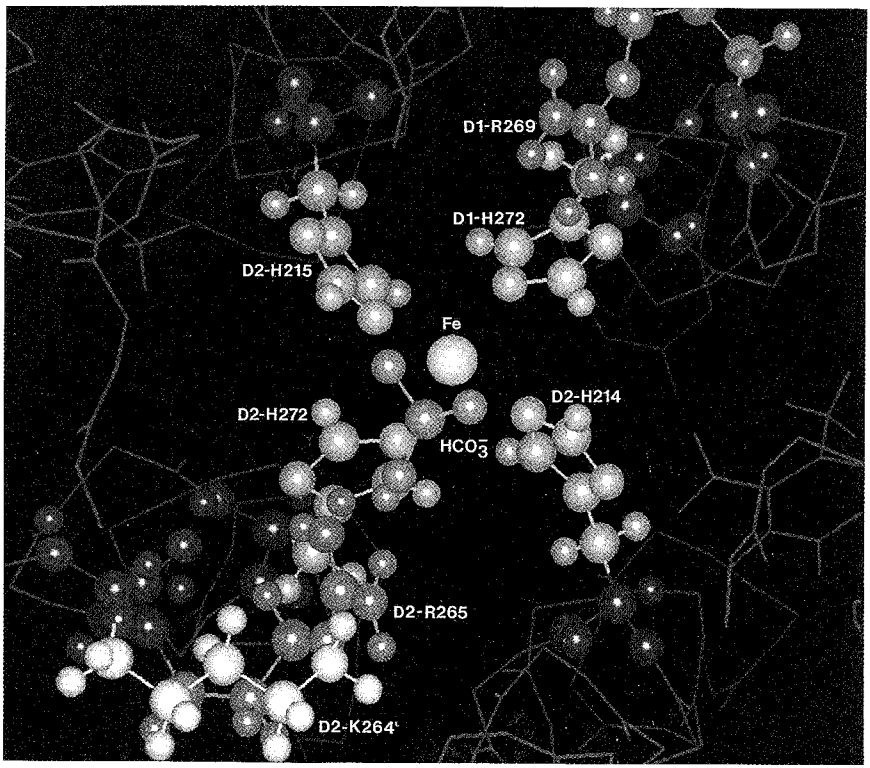

FIGURE 8 A possible site for bicarbonate (HCO_3^-) binding near the non-heme iron between the D1 and D2 polypeptides of the reaction center II. In this diagram, certain key amino acids (histidine, H; arginine, R; and lysine, K) are shown also (C. Gibas and Govindjee, unpublished data).

V. Mechanism of the bicarbonate effect in photosystem II

The major site of impairment by CO_2 depletion and formate or NO addition is now well established to be on the electron acceptor side of PSII. In the two preceding sections, evidence was summarized that indicated that the binding niche of formate or bicarbonate is located between helices D and E and the parallel helix DE of the D1 and D2 proteins. In this section, results are discussed that give additional information on the mechanism of the bicarbonate effect.

A. Back reactions of photosystem II and thermoluminescence

Thermoluminescence reflects mainly PSII reactions and has proved to be a useful technique to probe these reactions in thylakoids and in leaves (Rutherford *et al.*, 1984; Demeter and Govindjee, 1989). Govindjee *et al.* (1984) measured thermoluminescence from isolated thylakoids to probe the recombination reactions of the redox states of the water oxidation complex, for example, S_2 (or possibly S_3) with Q_B^- or Q_A^-, after CO_2-depletion treatment and readdition of bicarbonate. The effects of prolonged CO_2-depletion treatment on the $S_2Q_B^-$ (or $S_3Q_B^-$) thermoluminescence band were: (1) a 6–10°C shift to a higher temperature; (2) a reduction in intensity; and (3) elimination after the first few flashes of the characteristic period four oscillations in its intensity as a function of the flash number. These effects were fully reversible on addition of bicarbonate. Addition of diuron (DCMU) produced the same thermoluminescence band, at about +20°C, and was assigned to $S_2Q_A^-$ recombination in both CO_2-depleted and reconstituted samples. The initial effect of CO_2 depletion was concluded to be an increase in the activation energy for $S_2(S_3)Q_B^-$ recombination. With further depletion, the incidence of this recombination decreases so the cycling of $S_2Q_B^-$ and $S_3Q_B^-$ recombination is inhibited through effects on the D1 protein. Thermoluminescence data obtained after continuous light illumination suggest an increase in $S_2Q_A^-$ recombination at the expense of $S_2Q_B^-$ recombinations on removal of CO_2 from leaves (Garab *et al.*, 1988). A retardation in the electron flow between Q_A and Q_B may suffice to explain these data.

B. Inhibitors used to remove CO_2; uniqueness of bicarbonate

The inhibitor most often used to remove bicarbonate from its binding site is formate. Govindjee *et al.* (1991c) showed that formate removes bound CO_2 from thylakoid membranes. Stemler and Murphy (1985) reported inhibition of PSII electron transport by F^-, HCO_2^-, NO_2^-, and $CH_3CO_2^-$. All these inhibitions were reversed by the addition of bicarbonate. This class of anions

competitively inhibited bicarbonate binding to PSII. All the anions tested reduced bicarbonate binding more in the light than in the dark (Jursinic and Stemler, 1988). Cao and Govindjee (1990) found that a weak acid anion azide also inhibits PSII electron flow and that this inhibition is reversed by bicarbonate addition as well.

Diner and Petrouleas (1990) introduced nitric oxide (NO) as an inhibitor. They showed that, in spinach thylakoids, NO, like formate, slows electron transfer between the primary (Q_A) and secondary (Q_B) electron acceptors of PSII. In a series of saturating flashes given to dark-adapted thylakoids treated with NO, this electron transfer is slowed by at least a factor of 10 after the second and subsequent saturating flashes, compared with untreated thylakoids. This slowing effect is reversed completely by the addition of 10 m*M* bicarbonate, suggesting that bicarbonate displaces NO, like formate, from its binding site.

In all mentioned cases of inhibition of electron flow, reactivation of the PSII electron flow was possible by addition of *only* bicarbonate. Thus, this compound appears to have a special function on the reducing side of PSII when bound.

C. Active species: CO_2, HCO_3^-, or CO_3^{2-}?

Because formate and acetate were particularly effective in evoking a bicarbonate effect, Good (1963) suggested that the bicarbonate ion, not CO_2, is the important species. This idea was supported by Stemler and Govindjee (1973), who demonstrated a larger stimulation of the Hill reaction at pH 6.8 than at pH 5.8. The pH profile of the bicarbonate dependence shows an optimum around pH 6.5 (Khanna *et al.*, 1977; Vermaas and Van Rensen, 1981). Because of the close proximity of the pH optimum to the pK_a of HCO_3^-/CO_2 ($pK_a = 6.4$), one could consider the possibility that both CO_2 and bicarbonate are involved.

Since CO_2 is uncharged and nonpolar, it is expected to be a diffusing species (Sarojini and Govindjee, 1981). However, it would not be expected to bind to an active site other than by covalent attachment, whereas the observed interaction between inhibitory anions and bicarbonate is more consistent with an anionic binding. Thus, bicarbonate is thought to be more likely to be the active species. Observations by Xu *et al.* (1991) that formic acid, not formate, could be the inhibitory species challenges this premise and raises additional questions about the nature of the binding niche. CO_2 can form relatively unstable carbamate complexes with protein amino groups, which decompose readily. Such carbamate formation, for instance, has been demonstrated for the regulation of ribulose 1,5-bisphosphate carboxylase by CO_2 (Lorimer *et al.*, 1976). However, that bicarbonate is the active species at

equilibrium was demonstrated by taking advantage of the pH dependence of the equilibrium ratio of $[CO_2]$ to $[HCO_3^-]$ to effectively hold one concentration constant while varying the other (Blubaugh and Govindjee, 1986). The restoration of the Hill reaction was shown to be dependent only on $[HCO_3^-]$ and was apparently independent of $[CO_2]$, $[H_2CO_3]$, or $[CO_3^{2-}]$ over the pH range studied (6.3 - 6.9), which spanned both sides of the pH optimum. Although these results indicate that bicarbonate is the binding species under equilibrium conditions, they do not disprove a possible role for CO_2.

D. Binding affinity and the number of binding sites

Stemler (1977) measured binding of $H^{14}CO_3^-$ to isolated thylakoids and determined that there were two pools of bicarbonate: a high-affinity pool at a concentration of approximately 1 bicarbonate per 300 - 400 Chl molecules and a low-affinity pool at a concentration as large as or larger than that of the bulk Chl. Depletion of the high-affinity pool was correlated with the loss of Hill activity, whereas the role of the low-affinity pool, presumed to be largely empty under physiological conditions, remained undetermined.

In the high-affinity pool, two cooperative binding sites were suggested for bicarbonate binding, as mentioned earlier (Blubaugh, 1987; Blubaugh and Govindjee, 1988b).

E. Multiple effects

Punnett and Iyer (1964) observed that, by adding relatively high concentrations of bicarbonate to non-CO_2-depleted chloroplasts, they could accelerate the Hill reaction as well as the rate of phosphorylation. One of the effects of added bicarbonate appeared to be improved coupling between electron transport and phosphorylation. This bicarbonate effect is different from that on uncoupled PSII electron transport discussed earlier in this chapter. Differences between these two effects were reviewed by Govindjee and Van Rensen (1978) and by Blubaugh and Govindjee (1988a).

Although most of the experiments on PSII electron flow mentioned thus far suggest an effect of bicarbonate beyond Q_A, some experiments suggest that a bicarbonate-reversible inhibition also occurs prior to Q_A (see, e.g., El-Shintinawy *et al.*, 1990). Whether this effect is between the electron donor Z and the reaction center P680 or between Phe and Q_A remains to be seen.

F. Stabilization and protonation reactions

The steps at which protonation of Q_B occurs are not yet fully elucidated. Fowler (1977) observed a rather weak binary oscillation in proton uptake

during the production of plastoquinol (see also Van Rensen *et al.,* 1988). To account for an unprotonated Q_B^-, Förster *et al.* (1981) proposed the protonation of a protein group to stabilize Q_B^-, as was proposed earlier for photosynthetic bacteria (Wraight, 1979). It is tempting to assign a role to bicarbonate in this protonation since absence of bicarbonate slows the reduction of Q_B and the pK_a of CO_2/HCO_3^- is about 6.4. Such a speculation was made earlier (Govindjee and Van Rensen, 1978; Blubaugh and Govindjee, 1988a; Van Rensen *et al.,* 1988). However, bicarbonate is not likely to be the group that undergoes the pK_a shift observed by Crofts *et al.* (1984). For one thing, at pH values below 6.4 the putative protein group must be protonated already before Q_B^- formation, and Q_A^- to Q_B^- electron transfer must not be impaired (Crofts *et al.,* 1984). If bicarbonate itself were protonated, it would decompose to form CO_2, which would leave the bicarbonate site empty, so electron transfer from Q_A^- to Q_B^- would be impaired. However, bicarbonate could be responsible for providing a ready proton to an amino acid residue, as suggested by Blubaugh and Govindjee (1988a). Since bicarbonate-reversible inhibitors (e.g., formate) show a much smaller effect on the Q_A^- to Q_B reaction after the first flash than on Q_A^- to Q_B^- reaction after the second flash, it is logical to assume that this effect is on the protonation that leads to the stabilization of Q_B^- (Eaton-Rye and Govindjee, 1988; Xu *et al.,* 1991; Fig. 9).

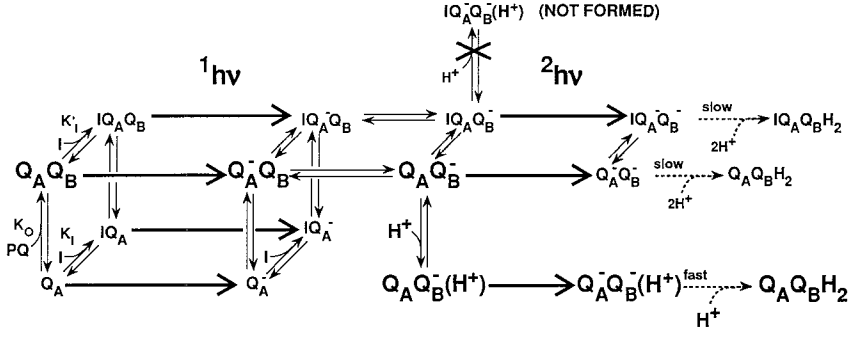

FIGURE 9 Scheme showing an inhibitor (I) binding mechanism that affects the two-electron gate in PSII reaction centers. This inhibitor effect is reversed completely by bicarbonate. Inhibitors include formate/formic acid, azide, nitrite, and nitric oxide. Boldface arrows denote light reactions after first flash ($^1h\nu$) or after second flash ($^2h\nu$). The pathway with Q_A and Q_B boldfaced represents the pathway in the presence of bicarbonate or when H^+ is plentiful. The reversible arrows represent equilibria for dark reactions. K_O, K_I, and K'_I are association constants for plastoquinone (PQ) and inhibitors in the absence and the presence of Q_B. The X implies the blockage of a protonation reaction of Q_B^-. (Redrawn from Xu *et al.,* 1991.)

G. Model

As a working model, one bicarbonate (site A) may be proposed to be a bidentate ligand to the non-heme iron between Q_A and Q_B, forming a salt bridge that is necessary for the functional configuration of the RC. Perhaps this bicarbonate is also H-bonded to appropriate amino acid residues (still to be deciphered) on the D1 and D2 proteins. Disruption of this salt bridge, obtained under prolonged CO_2-depletion treatment, is suggested to alter the distance between Q_A and Q_B, resulting in a slower electron transfer that makes Q_A^- more accessible to direct oxidation and that alters the binding affinity of plastoquinone to the Q_B site.

A second high-affinity bicarbonate (site B) may be involved also; bicarbonate may bind to positively charged amino acids such as arginines in the D1 and D2 proteins (Blubaugh and Govindjee, 1988a). This binding may be involved in protonating a histidine near the Q_B binding site. The histidine could contain the group whose pK_a has been observed to shift from 6.4 to 7.9 on formation of Q_B^- (Crofts *et al.*, 1984). This pK_a shift, induced by the negative charge on Q_B^-, encourages protonation of the histidine by the bicarbonate, whose own pK_a is lowered by the electron-withdrawing effects of the arginine. Three possible Arg/His pairs were suggested to be involved (Blubaugh and Govindjee, 1988a). In two of these (D1-R257/H252 and D1-R269/H272), the arginine and histidine would be separated by a single helical turn whereas in the third (D1-R225/H215) they would be separated by two helical turns. An examination of D1 and D2 amino acid sequences (Figs. 4, 6) and a possible model of the Q_A-Fe-Q_B binding niche (Fig. 8) may suggest other possibilities for HCO_3^- binding. Bicarbonate ions at sites A and B may bind cooperatively. Results of site-directed mutagenesis of selected conserved amino acids between helix D and E of both D1 and D2 proteins are expected to provide the final working model.

VI. Summary and conclusions

At physiological pH, bicarbonate appears to H-bond to several amino acids on both D1 and D2 proteins, the RC subunits of PSII. Bicarbonate may form a ligand to the non-heme iron that lies between Q_A (bound to D2) and Q_B (bound to D1). Bicarbonate, at physiological pH, is suggested to play a discrete and unique role(s) in PSII: its function may involve stabilization, by conformational means, of the RC protein that allows efficient electron flow and protonation of certain amino acids near Q_B^-. When the main function of HCO_3^- is to act as a proton conduit, it may become unnecessary at low pH value.

Data on differential sensitivity of the bicarbonate-reversible formate effect on the D1 protein mutants of *Chlamydomonas reinhardtii* and of *Synechocystis* 6714 strongly support the idea that the binding domains of bicarbonate are located between helices D and E and in the connecting DE loop of D1. Data on the sensitivity of D2-R233Q, D2-R251S, D2-K264X, and D2-R265X mutants to bicarbonate-reversible formate inhibition of electron flow in *Synechocystis* sp. PCC 6803 suggest that positively charged amino acids arginine and lysine may stabilize bicarbonate on the RC II.

Absence of the bicarbonate effect in purple photosynthetic bacteria and in green bacteria suggests that we should concentrate on the unique differences between the PSII (D1 and D2) and the bacterial (L and M) proteins to unravel the binding niche of bicarbonate and, thus, its function.

Acknowledgments

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References

Andersson, B., and Styring, S. (1991). Photosystem II: molecular organization, function and acclimation. *Curr. Top. Bioenerg.* **16,** 1-81.

Andreasson, L. E., and Vanngard, T. (1988). Electron transport in photosystems I and II. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39,** 379-411.

Barber, J. (1988). Similarities and differences between the photosystem two and purple bacterial reaction centers. *In* "Light Energy Transduction: Higher Plant and Bacterial Models" (S. E. Stevens, Jr., and D. Bryant, eds.), pp. 178-196. American Society of Plant Physiologists, Rockville, Maryland.

Bixon, J., Jortner, J., and Michel-Beyerle, M. E. (1991). On the mechanism of the primary charge separation in bacterial photosynthesis. *Biochim. Biophys. Acta* **1056,** 301-315.

Blankenship, R. E., Trost, J. T., and Manchino, L. E. (1987). Properties of reaction centers from the green photosynthetic bacterium *Chloroflexus aurantiacus. In* "The Photosynthetic Reaction Center: Structure and Dynamics" (J. Breton and A. Vermeglio, eds.), pp. 119-127. Plenum Press, New York.

Blubaugh, D. J. (1987). The mechanism of bicarbonate activation of plastoquinone reduction in photosystem II of photosynthesis. Ph.D. Thesis. University of Illinois, Urbana-Champaign.

√ Blubaugh, D. J., and Govindjee (1986). Bicarbonate, not CO_2, is the species required for the stimulation of photosystem II electron transport. *Biochim. Biophys. Acta* **848,** 147-152.

√ Blubaugh, D. J., and Govindjee (1988a). The molecular mechanism of the bicarbonate effect at the plastoquinone reductase site of photosynthesis. *Photosynth. Res.* **19,** 85-128.

√ Blubaugh, D. J., and Govindjee (1988b). Kinetics of the bicarbonate effect and the number of bicarbonate-binding sites in thylakoid membranes. *Biochim. Biophys. Acta* **936,** 208-214.

√ Blubaugh, D. J., and Govindjee (1988c). Sites of inhibition by disulfiram in thylakoid membranes. *Plant Physiol.* **88,** 1021-1025.

Bouges-Bocquet, B. (1973). Electron transfer between the two photosystems in spinach chloroplasts. *Biochim. Biophys. Acta* **314,** 250-256.

Bowden, S. J., Hallahan, B. J., Ruffle, S. V., Evans, M. C. W., and Nugent, J. H. A. (1991). Preparation and characterization of photosystem II core particles with and without bound bicarbonate. *Biochim. Biophys. Acta* **1060,** 89-96.

Brettel, K., Schlodder, E., and Witt, H. T. (1984). Nanosecond reduction kinetics of photooxidized chlorophyll α_{II} (P680) in single flashes as a probe for the electron pathway, H^+ release and charge accumulation in the O_2-evolving complex. *Biochim. Biophys. Acta* **766,** 403-428.

Burnap, R. L., and Sherman, L. A. (1991). Deletion mutagenesis in *Synechocystis* sp. PCC 6803 indicates that the Mn-stabilizing protein of photosystem II is not essential for O_2 evolution. *Biochemistry* **30,** 440-446.

Cao, J., and Govindjee (1988). Bicarbonate effect on electron flow in a cyanobacterium *Synechocystis* sp. PCC 6803. *Photosynth. Res.* **19,** 277-285.

Cao, J., and Govindjee (1990). Anion effects on the electron acceptor side of photosystem II in a transformable cyanobacterium *Synechocystis* 6803. *Curr. Res. Photosynth.* **I,** 515-518.

Cao, J., Vermaas, W. F. J., and Govindjee (1991). Arginine residues in the D2 polypeptide may stabilize bicarbonate binding in photosystem II of *Synechocystis* sp. PCC 6803. *Biochim. Biophys. Acta* **1059,** 171-180.

Cao, J., Ohad, N., Hirschberg, J., Xiong, J., and Govindjee (1992). Binding affinity of bicarbonate and formate in herbicide-resistant D1 mutants of *Synechococcus* sp. PCC 7942. *Photosynth. Res.* **34,** 397-408.

Cho, H. M., Mancino, L. J., and Blankenship, R. E. (1984). Light saturation curves and quantum yields in reaction centers from photosynthetic bacteria. *Biophys. J.* **45,** 455-461.

Coleman, W., and Govindjee (1987). A model for the mechanism of chloride activation of oxygen evolution in photosystem II. *Photosynth. Res.* **13,** 199-223.

Crofts, A. R., and Wraight, C. A. (1983). The electrochemical domain of photosynthesis. *Biochim. Biophys. Acta* **726,** 149-185.

Crofts, A. R., Robinson, H. H., and Snozzi, M. (1984). Reactions of quinones at catyalytic sites; a diffusional role in H-transfer. *In* "Advances in Photosynthesis Research" (C. Sybesma, ed.), pp. 461-468. Nijhoff, Dordrecht, The Netherlands.

Debus, R., Barry, B. A., Sithole, I., Babcock, G. T., and McIntosh, L. (1988a). Directed mutagenesis indicates that the donor to $P680^+$ in photosystem II is tyrosine-161 of the D1 polypeptide. *Biochemistry* **27,** 9071-9074.

Debus, R., Barry, B. A., Babcock, G. T., and McIntosh, L. (1988b). Site-directed mutagenesis identifies a tyrosine radical in the photosynthetic oxygen-evolving system. *Proc. Natl. Acad. Sci. U.S.A.* **85,** 427-430.

Deisenhofer, J., and Michel, H. (1989). The photosynthetic reaction centre from the purple bacterium *Rhodopseudomonas viridis*. *EMBO J.* **8,** 2149-2169.

Deisenhofer, J., Epp, O., Miki, K., Huber, R., and Michel, H. (1985). Structure of the protein subunits in the photosynthetic reaction centre of *Rhodopseudomonas viridis* at 3 Å resolution. *Nature (London)* **318,** 618-624.

Demeter, S., and Govindjee (1989). Thermoluminescence from plants. *Physiol. Plant.* **75,** 121-130.

Diner, B. A., and Petrouleas, V. (1987). Q_{400}, the non-heme iron of the photosystem II iron-quinone complex. A spectroscopic probe of quinone and inhibitor binding to the reaction center. *Biochim. Biophys. Acta* **895,** 107-125.

Diner, B. A., and Petrouleas, V. (1990). Formation by NO of nitrosyl adducts of redox components of the photosystem II reaction center. II. Evidence that HCO_3^-/CO_2 binds to the acceptor-side non-heme iron. *Biochim. Biophys. Acta* **1015,** 141-149.

Diner, B. A., Petrouleas, V., and Wendoloski, J. J. (1991). The iron-quinone electron-acceptor complex of photosystem II. *Physiol. Plant.* **81,** 423-436.

Eaton-Rye, J. J., and Govindjee (1984). A study of the specific effect of bicarbonate on photosynthetic electron transport in the presence of methyl viologen. *Photobiochem. Photobiophys.* **8,** 279 - 288.

Eaton-Rye, J. J., and Govindjee (1988). Electron transfer through the quinone acceptor complex of photosystem II in bicarbonate-depleted spinach thylakoid membranes as a function of actinic flash number and frequency. *Biochim. Biophys. Acta* **935,** 237 - 257.

Eckert, H. J., Weise, N., Bernarding, J., Eichler, H. J., and Renger, G. (1988). Analysis of the electron transfer from $Pheo^-$ to Q_A in PSII membrane fragments from spinach by time resolved 325 nm absorbance changes in the picosecond domain. *FEBS Lett.* **240,** 153 - 158.

El-Shintinawy, F., Xu, C., and Govindjee (1990). A dual bicarbonate-reversible formate effect in *Chlamydomonas* cells. *J. Plant Physiol.* **136,** 421 - 428.

Etienne, A.-L., Ducruet, J.-M., Ajlani, G. K., and Vernotte, C. (1990). Comparative studies on electron transfer in photosystem II of herbicide-resistant mutants from different organisms. *Biochim. Biophys. Acta* **1015,** 435 - 440.

Feher, G., Allen, J. P., Okamura, M. Y., and Rees, D. C. (1989). Structure and function of bacterial photosynthetic reaction centers. *Nature (London)* **339,** 111 - 116.

Förster, V., Hong, Y.-Q., and Junge, W. (1981). Electron transfer and proton pumping under excitation of dark-adapted chloroplasts with flashes of light. *Biochim. Biophys. Acta* **638,** 141 - 152.

Fowler, C. F. (1977). Proton translocation in chloroplasts and its relationship to electron transport between the photosystems. *Biochim. Biophys. Acta* **459,** 351 - 363.

Garab, G., Rozsa, Z., and Govindjee (1988). Carbon dioxide affects charge accumulation in leaves. *Naturwiss.* **75,** 517 - 519.

Gerken, S., Brettel, K., Schlodder, E., and Witt, H. T. (1988). Optical characterization of the immediate electron donor to chlorophyll α_{II}^+ in O_2-evolving photosystem II complexes. Tyrosine as possible electron carrier between chlorophyll α_{II} and the water-oxidizing manganese complex. *FEBS Lett.* **237,** 69 - 75.

Ghanotakis, D. F., and Yocum, C. F. (1990). Photosystem II and the oxygen-evolving complex. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **41,** 255 - 276.

Good, N. E. (1963). Carbon dioxide and the Hill reaction. *Plant Physiol.* **38,** 298 - 304.

Govindjee (1991). A unique role of carbon dioxide in photosystem II. *In* "Impact of Global Climatic Changes on Photosynthesis and Plant Productivity" (Y. Abrol, P. N. Wattal, A. Gnanam, Govindjee, D. R. Ort, and A. H. Teramura, eds.), pp. 349 - 369. Oxford/IBH, New Delhi, India.

Govindjee, and Eaton-Rye, J. J. (1986). Electron transfer through photosystem II acceptors: interaction with anions. *Photosynth. Res.* **10,** 365 - 379.

Govindjee, and Govindjee, R. (1975). Introduction to photosynthesis. *In* "Bioenergetics of Photosynthesis" (Govindjee, ed.), pp. 1 - 50. Academic Press, New York.

Govindjee, and Van Rensen, J. J. S. (1978). Bicarbonate effects on the electron flow in isolated broken chloroplasts. *Biochim. Biophys. Acta* **505,** 183 - 213.

Govindjee, Pulles, M. J. P., Govindjee, R., Van Gorkom, H. J., and Duysens, L. N. M. (1976). Inhibition of the reoxidation of the secondary electron acceptor of photosystem II by bicarbonate depletion. *Biochim. Biophys. Acta* **449,** 602 - 605.

Govindjee, Nakatani, H. Y., Rutherford, A. W., and Inoue, Y. (1984). Evidence from thermoluminescence for bicarbonate action on the recombination reactions involving the secondary quinone electron acceptor of photosystem II. *Biochim. Biophys. Acta* **766,** 416 - 423.

Govindjee, Vernotte, C., Peteri, B., Astier, C., and Etienne, A.-L. (1990). Differential sensitivity of bicarbonate-reversible formate effects on herbicide-resistant mutants of *Synechocystis* 6714. *FEBS Lett.* **267,** 273 - 276.

Govindjee, Trost, J. F., and Blankenship, R. E. (1991a). Formate and nitric oxide fail to inhibit electron flow in reaction centers from green bacterium *Chloroflexus aurantiacus*. *Photochem. Photobiol.* **53** (supplement) 121S - 122S.

Govindjee, Schwarz, B., Rochaix, J.-D., and Strasser, R. J. (1991b). A herbicide-resistant mutant L275F of *Chlamydomonas reinhardtii* fails to show the bicarbonate-reversible formate effect in chlorophyll *a* fluorescence transients. *Photosynth. Res.* **27,** 199-208.

Govindjee, Weger, H. G., Turpin, D., van Rensen, J. J. S., de Vos, O. J., and Snel, J. F. H. (1991c). Formate releases carbon dioxide/bicarbonate from thylakoid membranes: measurements by mass spectroscopy and infrared gas analyzer. *Naturwiss.* **78,** 168-170.

Hansson, O., and Wydrzynski, T. (1990). Current perceptions of photosystem II. *Photosynth. Res.* **23,** 131-162.

Holzapfel, W., Finkele, U., Kaiser, W., Oesterhelt, D., Scheer, H., Stilz, H. U., and Zinth, W. (1990). Initial electron-transfer in the reaction center from *Rhodobacter sphaeroides*. *Proc. Natl. Acad. Sci. U.S.A.* **87,** 5168-5172.

Ikeuchi, M. (1992). Subunit proteins of photosystem II. *Bot. Mag. Tokyo* **105,** 327-373.

Ikeuchi, M., Koike, H., and Inoue, Y. (1989). Identification of *psbI* and *psbL* gene products in cyanobacterial photosystem II reaction center preparation. *FEBS Lett.* **251,** 155-160.

Jursinic, P., and Govindjee (1977). Temperature dependence of delayed light emission in the 6 to 340 μs range after a single flash in chloroplasts. *Photochem. Photobiol.* **26,** 617-628.

Jursinic, P., and Stemler, A. (1988). Multiple anion effects on photosystem II in chloroplast membranes. *Photosynth. Res.* **15,** 41-56.

Jursinic, P., Warden, J., and Govindjee (1976). A major site of bicarbonate effect in system II reaction: evidence from ESR signal II_{vf}, fast fluorescence yield changes and delayed light emission. *Biochim. Biophys. Acta* **440,** 322-330.

Khanna, R., Govindjee, and Wydrzynski, T. (1977). Site of bicarbonate effect in Hill reaction. Evidence from the use of artificial electron acceptors and donors. *Biochim. Biophys. Acta* **462,** 208-214.

Khanna, R., Pfister, K., Keresztes, A., Van Rensen, J. J. S., and Govindjee (1981). Evidence for a close spatial location of the binding sites for CO_2 and for photosystem II inhibitors. *Biochim. Biophys. Acta* **634,** 105-116.

Kobayashi, M., Maeda, H., Watanabe, T., Nakane, H., and Satoh, K. (1990). Chlorophyll a and β-carotene content in the D1/D2/cytochrome *b*559 reaction center complex from spinach. *FEBS Lett.* **260,** 138-140.

Lorimer, G. H., Badger, M. R., and Andrews, T. J. (1976). The activation of ribulose-1,5-bisphosphate carboxylase by carbon dioxide and magnesium ions: equilibria, kinetics, a suggested mechanism, and physiological implications. *Biochem. J.* **15,** 529-536.

Mayes, S. R., Cook, K. M., Self, S. J., Zhang, Z., and Barber, J. (1991). Deletion of the gene encoding the photosystem II 33 kDa protein from *Synechocystis* sp. PCC 6803 does not inactivate water-splitting but increases vulnerability to photoinhibition. *Biochim. Biophys. Acta* **1060,** 1-12.

Metz, J. G., Nixon, P. J., Rogner, M., Brudvig, G. W., and Diner, B. A. (1989). Directed alteration of the D1 polypeptide of photosystem II; Evidence that tyrosine-161 is the redox component, Z, connecting the oxygen-evolving complex to the primary electron donor, P680. *Biochemistry* **28,** 6960-6969.

Michel, H., and Deisenhofer, J. (1988). Relevance of the photosynthetic reaction center from purple bacteria to the structure of photosystem II. *Biochemistry* **27,** 1-7.

Moenne-Loccoz, P., Robert, B., and Lutz, M. (1989). A resonance Raman characterization of the primary electron acceptor in photosystem II. *Biochemistry* **28,** 3641-3645.

Nabedryk, E., Andrianambinintsoa, S., Berger, G., Leonard, M., Mantele, W., and Breton, J. (1990). Characterization of bonding interactions of the intermediary electron acceptor in the reaction center of photosystem II by FTIR spectroscopy. *Biochim. Biophys. Acta* **1016,** 49-54.

Naber, J. D., and Van Rensen, J. J. S. (1991). Activity of photosystem II herbicides is related with their residence times at the D1 protein. *Z. Naturforsch.* **46c,** 49-52.

Nixon, P. J., Chisholm, D. A., and Diner, B. A. (1992). Isolation and functional analysis of random

and site-directed mutants for photosystem II. *In* "Plant Protein Engineering" (P. Shewry and S. Gutteridge, eds.), pp. 93-141. Cambridge University Press, Cambridge.

Norris, J. R., and Schiffer, M. (1990). Photosynthetic reaction centers in bacteria. *Chem. Eng. News* **July 30,** 22-37.

Nugent, J. H. A., Corrie, A. R., Demetriou, C., Evans, M. C. W., and Lockett, C. J. (1988). Bicarbonate binding and the properties of photosystem II electron acceptors. *FEBS Lett.* **235,** 71-75.

Nuijs, A. M., Van Gorkom, H. J., Plijter, J. J., and Duysens, L. N. M. (1986). Primary charge separation and excitation of chlorophyll *a* in photosystem II particles from spinach as studied by picosecond absorbance difference spectroscopy. *Biochim. Biophys. Acta* **848,** 167-175.

Oettmeier, W. (1992). Herbicides of photosystem II. *In* "Topics in Photosynthesis, Vol. 11: The Photosystems: Structure, Function and Molecular Biology" (J. Barber, ed.), pp. 349-404. Elsevier Science Publishers, Amsterdam, The Netherlands.

Ovchinikov, Y., Abdulaev, N. G., Zolotarev, A. S., Shmuckler, B. E., Zargarov, A. A., Kutuzov, M. A., Telezhinskaya, I. N., and Levina, N. B. (1988a). Photosynthetic reaction centre of *Chloroflexus aurantiacus.* I. Primary structure of L-subunit. *FEBS Lett.* **231,** 237-241.

Ovchinikov, Y., Abdulaev, N. G., Shmuckler, B. E., Zargarov, A. A., Kutuzov, M. A., Telzhinskaya, I. N., Levina, N. B., and Zolotarev, A. S. (1988b). Photosynthetic reaction centre of *Chloroflexus aurantiacus.* II. Primary structure of M-subunit. *FEBS Lett.* **232,** 364-368.

Pakrasi, H., and Vermaas, W. F. J. (1992). Protein engineering of photosystem II. *In* "Topics in Photosynthesis, Vol. 11: The Photosystems: Structure, Function and Molecular Biology" (J. Barber, ed.), pp. 231-257. Elsevier Science Publishers, Amsterdam, The Netherlands.

Petrouleas, V., and Diner, B. (1986). Identification of Q_{400}, a high potential electron acceptor of photosystem II, the iron of the quinone-iron acceptor complex. *Biochim. Biophys. Acta* **849,** 264-275.

Petrouleas, V., and Diner, B. A. (1990). Formation by NO of nitrosyl adducts of redox components of the photosystem II reaction center. I. NO binds to the acceptor-side non-heme iron. *Biochim. Biophys. Acta* **1015,** 131-140.

Philbrick, J. B., Diner, B. A., and Zilinskas, B. A. (1991). Construction and characterization of cyanobacterial mutants lacking the manganese-stabilizing polypeptide of photosystem II. *J. Biol. Chem.* **226,** 133.

Punnett, T., and Iyer, R. V. (1964). The enhancement of photophosphorylation and the Hill reaction by carbon dioxide. *J. Biol. Chem.* **239,** 2335-2339.

Renger, G. (1992). Energy transfer and trapping in photosystem II. *In* "Topics in Photosynthesis, Vol. 11: The Photosystems: Structure, Function and Molecular Biology" (J. Barber, ed.), pp. 45-99. Elsevier Science Publishers, Amsterdam, The Netherlands.

Robinson, H. H., Eaton-Rye, J. J., Van Rensen, J. J. S., and Govindjee (1984). The effects of bicarbonate depletion and formate incubation on the kinetics of oxidation-reduction reactions of the photosystem II quinone acceptor complex. *Z. Naturforsch.* **39c,** 382-385.

Rutherford, A. W. (1988). Photosystem II, the oxygen evolving system. *In* "Light-Energy Transduction in Photosynthesis: Higher Plant and Bacterial Models" (S. E. Stevens, Jr., and D. A. Bryant, eds.), pp. 163-177. American Society of Plant Physiologists, Rockville, Maryland.

Rutherford, A. W., Govindjee, and Inoue, Y. (1984). Charge accumulation and photochemistry in leaves studied by thermoluminescence and delayed light emission. *Proc. Natl. Acad. Sci. U.S.A.* **81,** 1107-1111.

Sarojini, G., and Govindjee (1981). On the active species in bicarbonate stimulation of Hill reaction in thylakoid membranes. *Biochim. Biophys. Acta* **634,** 340-343.

Satoh, K. (1988). Reality of P-680 chlorophyll protein. Identification of the site of primary photochemistry in oxygenic photosynthesis. *Physiol. Plant.* **72,** 209-212.

Semin, B. K., Loviagina, E. R., Aleksandrov, A. Y., Kaurov, Y. N., and Novakova, A. A. (1990).

Effect of formate on Mossbauer parameters of the non-heme iron of PSII particles of cyanobacteria. *FEBS Lett.* **270,** 184-186.

Shipman, L. (1981). Theoretical study of the binding site and mode of action for photosystem II herbicides. *J. Theor. Biol.* **90,** 123-148.

Shopes, R. J., Blubaugh, D., Wraight, C., and Govindjee (1989). Absence of a bicarbonate-depletion effect in electron transfer between quinones and reaction centers of *Rhodobacter sphaeroides. Biochim. Biophys. Acta* **974,** 114-118.

Snel, J. F. H., and Van Rensen, J. J. S. (1983). Kinetics of the reactivation of the Hill reaction in CO_2-depleted chloroplasts by addition of bicarbonate in the absence and in the presence of herbicides. *Plant Physiol.* **57,** 422-427.

Snel, J. F. H., and Van Rensen, J. J. S. (1984). Reevaluation of the role of bicarbonate and formate in the regulation of photosynthetic electron flow in broken chloroplasts. *Plant Physiol.* **75,** 146-150.

Sonneveld, A., Rademaker, H., and Duysens, L. N. M. (1979). Chlorophyll *a* fluorescence as a monitor of nanosecond reduction of the photooxidized primary donor P-680$^+$ of photosystem II. *Biochim. Biophys. Acta* **548,** 536-551.

Stemler, A. (1977). The binding of bicarbonate ions to washed chloroplast grana. *Biochim. Biophys. Acta* **460,** 511-522.

Stemler, A. (1980). Inhibition of photosystem II by formate: possible evidence for a direct role of bicarbonate in photosynthetic oxygen evolution. *Biochim. Biophys. Acta* **593,** 103-112.

Stemler, A., and Govindjee (1973). Bicarbonate ion as a critical factor in photosynthetic oxygen evolution. *Plant Physiol.* **52,** 119-123.

Stemler, A., and Murphy, J. B. (1985). Bicarbonate-reversible and irreversible inhibition of photosystem II by monovalent anions. *Plant Physiol.* **77,** 974-977.

Stewart, A. C., Ljunberg, V., Åkerlund, H.-E., and Andersson, B. (1985). Studies on the polypeptide composition of the cyanobacterial oxygen-evolving complex. *Biochim. Biophys. Acta* **808,** 353-362.

Svensson, B., Vass, I., Cedergren, E., and Styring, S. (1990). Structure of donor side components in photosystem II predicted by computer modeling. *EMBO J.* **9,** 2051-2059.

Takahashi, E., and Wraight, C. A. (1990). A crucial role for Asp^{L213} in the protein transfer pathway to the secondary quinone of reaction centers from *Rhodobacter sphaeroides. Biochim. Biophys. Acta* **1020,** 107-111.

Trebst, A. (1986). The topology of the plastoquinone and herbicide binding peptides of photosystem II in the thylakoid membrane. *Z. Naturforsch.* **41c,** 240-245.

Trebst, A. (1991). The molecular basis of resistance of photosystem II herbicides. *In* "Herbicide Resistance in Weeds and Crops" (J. C. Caseley, G. W. Cussans, and R. K. Atkin, eds.), pp. 145-164. Butterworth-Heinemann, Oxford.

Van Best, J. A., and Mathis, P. (1978). Kinetics of the oxidized primary electron donor of photosystem II in spinach chloroplasts and in *Chlorella* cells in the microsecond and nanosecond time ranges following flash excitation. *Biochim. Biophys. Acta* **503,** 178-188.

Van Kan, P. J. M., Otte, S. C. M., Kleinerenbrink, F. A. M., Nieveen, M. C., Aartsma, T. J., and Van Gorkom, H. J. (1990). Time-resolved spectroscopy at 10K of the photosystem II reaction center; deconvolution of the red absorption band. *Biochim. Biophys. Acta* **1020,** 146-152.

Van Mieghem, F. J. E., Satoh, K., and Rutherford, A. W. (1991). A chlorophyll tilted 30° relative to the membrane in the photosystem II reaction centre. *Biochim. Biophys. Acta* **1058,** 379-385.

Van Rensen, J. J. S. (1969). Polyphosphate formation in *Scenedesmus* in relation to photosynthesis. *In* "Progress in Photosynthesis Research" (H. Metzner, ed.), Vol. III, pp. 1769-1776. H. Laupp, Jr. Tübingen.

Van Rensen, J. J. S. (1993). Regulation of electron transport at the acceptor side of photosystem

II by herbicides, bicarbonate and formate. *In* "Photosynthesis and Plant Productivity" (Y. Abrol, P. Mohanty, and Govindjee, eds.), pp. 157-180. Oxford/IBH, New Delhi, India.

Van Rensen, J. J. S., and Kramer, H. J. M. (1979). Short-circuit electron transport insensitive to diuron-type herbicides induced by treatment of isolated chloroplasts with trypsin. *Plant Sci. Lett.* **17,** 21-27.

Van Rensen, J. J. S., and Vermaas, W. F. J. (1981). Action of bicarbonate and photosystem II inhibiting herbicides on electron transport in pea grana and in thylakoids of a blue-green alga. *Physiol. Plant.* **51,** 106-110.

Van Rensen, J. J. S., Tonk, W. J. M., and De Bruin, S. M. (1988). Involvement of bicarbonate in the protonation of the secondary quinone electron acceptor of photosystem II via the non-heme iron of the quinone-iron acceptor complex. *FEBS Lett.* **226,** 347-351.

Velthuys, B. R. (1981). Electron dependent competition between plastoquinone and inhibitors for binding to photosystem II. *FEBS Lett.* **126,** 277-281.

Velthuys, B. R., and Amesz, J. (1974). Charge accumulation at the reducing side of system 2 of photosynthesis. *Biochim. Biophys. Acta* **333,** 85-94.

Vermaas, W. F. J., and Ikeuchi, M. (1991). Photosystem II. *In* "Photosynthetic Apparatus: Molecular Biology and Operation" (L. Bogorad and I. K. Vasil, eds.), pp. 25-111. Academic Press, San Diego.

Vermaas, W. F. J., and Rutherford, A. W. (1984). EPR measurements on the effects of bicarbonate and triazine resistance on the acceptor side of photosystem II. *FEBS Lett.* **175,** 243-248.

Vermaas, W. F. J., and Van Rensen, J. J. S. (1981). Mechanism of bicarbonate action on photosynthetic electron transport in broken chloroplasts. *Biochim. Biophys. Acta* **636,** 168-174.

Vermaas, W. F. J., Van Rensen, J. J. S., and Govindjee (1982). The interaction between bicarbonate and the herbicide ioxynil in the thylakoid membrane and the effects of amino acid modification on bicarbonate action. *Biochim. Biophys. Acta* **681,** 242-247.

Vermaas, W. F. J., Dohnt, G., and Renger, G. (1984). Binding and release kinetics of inhibitors of Q_A^- oxidation in thylakoid membranes. *Biochim. Biophys. Acta* **765,** 74-83.

Vermaas, W. F. J., Rutherford, A. W., and Hansson, Ö. (1988). Site-directed mutagenesis in photosystem II of the cyanobacterium *Synechocystis* sp. PCC 6803: Donor D is a tyrosyl residue in the D2 protein. *Proc. Natl. Acad. Sci. U.S.A.* **85,** 8477-8481.

Vermaas, W. F. J., Charité, J., and Shen, G. (1990). Glu-69 of the D2 protein in photosystem II is a potential ligand to Mn involved in photosynthetic oxygen evolution. *Biochemistry* **29,** 5325-5332.

Wang, X., Cao, J., Maroti, P., Stilz, H. U., Finkele, U., Lauterwasse, C. Zinth, W., Oesterhelt, D., Govindjee, and Wraight, C. A. (1992). Is bicarbonate in photosystem II the equivalent of the glutamate ligand to the iron atom in bacterial reaction centers? *Biochim. Biophys. Acta* **1100,** 1-8.

Warburg, O. (1964). Prefatory chapter. *Annu. Rev. Biochem.* **33,** 1-18.

Warburg, O., and Krippahl, G. (1958). Hill-Reaktionen. *Z. Naturforsch.* **13b,** 509-514.

Wasielewski, M. R., Johnson, D. G., Seibert, M., and Govindjee (1989). Determination of the primary charge separation rate in isolated photosystem II reaction centers with 500-fs time resolution. *Proc. Natl. Acad. Sci. U.S.A.* **86,** 524-528.

Wasielewski, M. R., Johnson, P. G., Govindjee, Preston, C., and Seibert, M. (1990). Determination of the primary charge separation rate in photosystem II reaction centers at 15 K. *Photosynth. Res.* **22,** 89-99.

Woese, C. R., Kandler, O., and Wheelis, M. L. (1990). Towards a natural system of organisms: proposal for the domains Archea, Bacteria and Eucarya. *Proc. Natl. Acad. Sci. U.S.A.* **87,** 4576-4579.

Wraight, C. A. (1979). Electron acceptors of bacterial photosynthetic reaction centers: II. H^+ binding coupled to secondary electron transfer in the quinone acceptor complex. *Biochim. Biophys. Acta* **548,** 309-327.

Wraight, C. A. (1981). Oxidation-reduction physical chemistry of the acceptor quinone complex

in bacterial photosynthetic reaction centers: evidence for a new model of herbicide activity. *Israel J. Chem.* **21,** 348-354.

Wydrzynski, T., and Govindjee (1975). A new site of bicarbonate effect in photosystem II of photosynthesis; evidence from chlorophyll fluorescence transients in spinach chloroplasts. *Biochim. Biophys. Acta* **387,** 403-408.

Xu, C., Taoka, S., Crofts, A. R., and Govindjee (1991). Kinetic characteristics of formate/formic acid binding at the plastoquinone reductase site in spinach chloroplasts. *Biochim. Biophys. Acta* **1098,** 32-40.

Zimmerman, J.-L., and Rutherford, A. W. (1986). Photoreductant-induced oxidation of Fe^{2+} in the electron acceptor complex of photosystem II. *Biochim. Biophys. Acta* **851,** 416-423.