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ELECTRON TRANSFER THROUGH PHOTOSYSTEM II ACCEPTORS: INTERACTION WITH ANIONS

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ABSTRACT

We present an overview of anionic interactions with the oxidation-reduction reactions of photosystem II (PSII) acceptors. In section 1, a framework is laid for the electron acceptor side of PSII: the overview begins with a current scheme of the electron transport pathway and of the localization of components in the thylakoid membrane, which is followed by a brief description of the electron acceptor Q or Q_A and the various heterogeneities associated with it. In section 2, we review briefly the nature of the active species of the bicarbonate (HCO_3^-) effect, the location of the site of action of HCO_3^-, and its relationship to interactions with other anions. In section 3, we review data on the anion effects on the reoxidation of Q_A^- and on the various reactions involved in the two-electron gate mechanism of PSII, and provide a hypothesis as to the action of HCO_3^- on the protonation reactions. New data obtained by one of us (G) in collaboration with J.J.S. van Rensen, J.F.H Snel and W. Tonk for HCO_3^--depleted thylakoids, demonstrating the abolition of the binary oscillations contained within the periodicity of 4 observed for proton release, are also reviewed. In section 4, we comment on the measured binding constant of HCO_3^- at the anion binding site. And, in section 5, we review our current concept of the mechanism of the HCO_3^- effect on the electron acceptor side of PSII, and comment on the possible physiological roles for HCO_3^-. Measurements of HCO_3^- reversible anionic inhibition in intact cells of a green alga Scenedesmus are also reviewed.

1. INTRODUCTION

Much of the information regarding the complexities of photosynthesis have been drawn from studies of the variable chlorophyll (Chl) a fluorescence yield [30]. Govindjee et al. [24] and Butler [6] showed that the variable fluorescence yield excited by PSII light could be quenched by simultaneous excitation by PSI light suggesting its relationship to a two photosystem-two light reaction scheme of photosynthesis. Kautsky et al. [44] explained the Chl a fluorescence transient in terms of the oxidation state of a member of the electron transport chain; fluorescence was suggested to be quenched when this component was oxidized by one light reaction, while its photochemical reduction by another light reaction gave rise to an increase in fluorescence. The designation of this acceptor as Q, for "quencher", arose from the work of Duysens [12] and Duysens and Sweers [13] (see Butler [7]). Q may be identified as Q_A, the primary quinone acceptor of PSII, in the electron transfer scheme of photosynthesis shown in Figs. 1 and 2. Figures 1 and 2, should, respectively, serve as a framework for electron transport, and the components, discussed in this book.

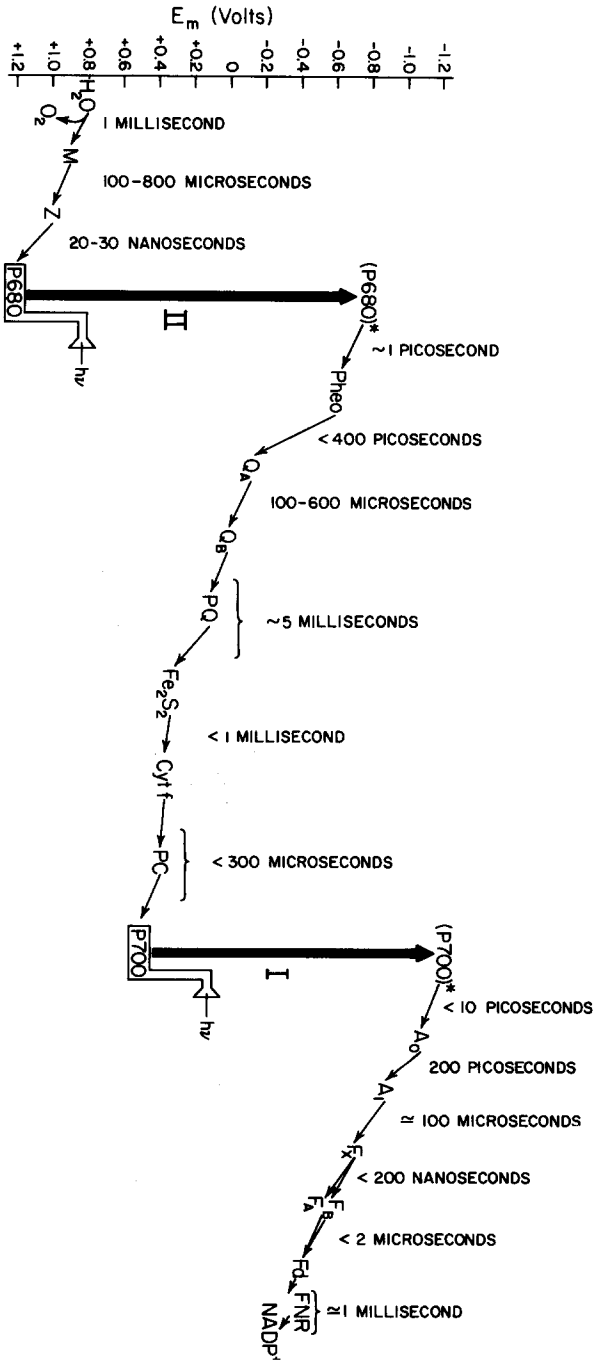

FIGURE 1. See legend on the following page.

FIGURE 1. Pathway of noncyclic electron flow from H_2O, the electron donor of photosynthesis, to nicotinamide adenine dinucleotide phosphate ($NADP^+$), the physiological electron acceptor. $E_{m,7}$ on the ordinate stands for mid-point redox potential. Light quanta ($h\nu$) are absorbed in two sets of antenna chlorophyll molecules, the excitation energy is transferred to the reaction center chlorophyll a molecules of photosystem II (P680) and photosystem I (P700) forming $\overline{P}680)^*$ and $(P700)^*$, and the latter two initiate electron transport. M stands for an all-purpose complex, the "M complex" or the oxygen evolving complex, but it specifically reflects the electron carriers that undergo redox reactions and charge accumulation; Z is the electron donor to P680; Pheo represents pheophytin; Q_A, Q_B and PQ are plastoquinone molecules (see Fig. 3); Fe_2S_2 represents the Rieske iron-sulphur center, Cyt f stands for cytochrome f, PC is plastocyanin; A_o is suggested to be a chlorophyll molecule, A_1 is possibly a quinone; F_A, F_B, and F_X are thought to be 4Fe-4S centers and FNR is ferredoxin NADP oxidoreductase. Estimated or directly measured times for various reactions are also indicated. In the case of PSII these are taken from [26] and for the PSI from [63]. The values for the intersystem chain are from [31]. In the case of PSI it has also been suggested that A_1 directly reduces F_A and/or F_B in approximately 200 ns while F_X reduction, in approximately 100 μs by A_1, represents a side pathway. For a detailed discussion see [63].

A heterogeneous population of electron acceptors seems to be present in PSII. Q does not represent a single chemical entity [2,85]: (1) Redox potentiometric titrations have revealed two components: Q_H which has an $E_{m,7}$ (mid-point potential at pH 7) of about 0 mV and Q_L which has an $E_{m,7}$ of about -250 mV [9,34]. (2) Parallel measurements on C550 (an absorbance change at 550 nm [16]) and variable Chl a fluorescence following single saturating flashes, in DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea)-treated samples, revealed the existence of two Q's: Q_1 and Q_2, where Q_1 was related to all of C550 and to 70% of variable fluorescence yield [37, 39]. Reduction of Q_H and Q_1 is associated with the creation of a membrane potential (ΔA 515), whereas reduction of Q_2 and Q_L is not [11,38]. Furthermore, Q_1 gives a semiquinone signal X-320, whereas Q_2 does not [40]. It appears that Q_1 and Q_H are the same acceptor located on a side different from that of Q_2 and Q_L.

Q_B, the secondary quinone acceptor of PSII (Figs. 1-3), is thought to function in a two-electron gating mechanism [5,83]. Electrons are first transferred from reduced pheophytin ($Pheo^-$) to Q_A, which can only be reduced to the semiquinone form. Q_A^- is then oxidized by Q_B (Fig. 3). After two such events, Q_B is reduced to plastoquinol ($Q_B^{2-}(2H^+)$) which then exchanges with a plastoquinone (PQ) from the plastoquinone pool (PQ pool). Independently, Velthuys [82] and Wraight [93] proposed that the mode of action of a number of PSII herbicides (e.g., DCMU in plants) is to compete with the quinone for the secondary acceptor binding site, the so-called B-site. Following a single actinic flash an equilibrium for an electron is set up between the two quinone acceptors. While Q_B and plastoquinol ($Q_B^{2-}(2H^+)$) are bound loosely at the B-site, Q_B^- is bound tightly and the equilibrium K_E (Fig. 3) is displaced towards $Q_B^-(H^+)$. A value of 20 for this parameter has been estimated at pH 7.6 [60]. In the presence of a non-electron accepting herbicide (I), such as DCMU, Q_A^-I is produced (Fig. 3); K_o and K_I are the association constants for Q_B and I respectively when

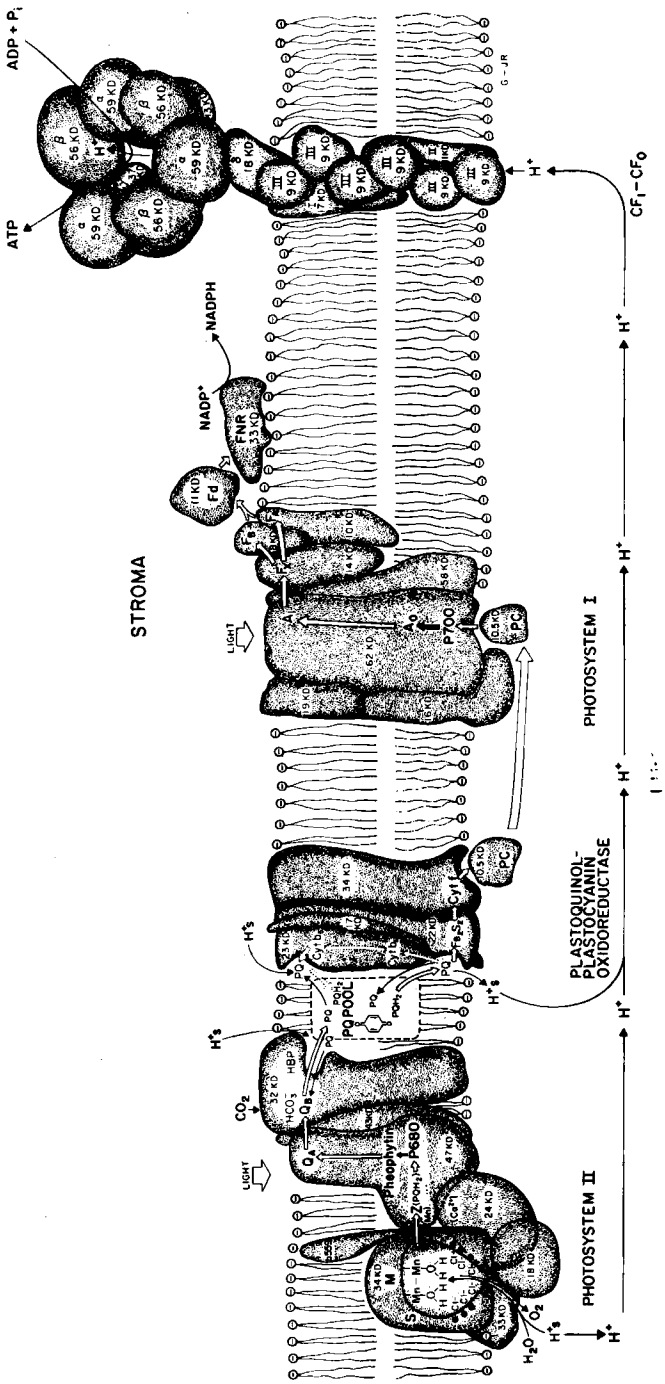

FIGURE 2. See legend on the following page.

FIGURE 2. A stylized model of the electron transport chain with the light-harvesting pigment-protein complexes omitted. The depiction of PSII is adapted from [26] and the organization of the plastoquinol-plastocyanin oxidoreductase or cytochrome $b_{6/f}$ complex is based on [52] and [59]. The organization of PSI is adapted from a recent overview given in [59] and the chapter by R. Malkin in this volume. The organization of the H^+-ATPase (CF_1-CF_0) is highly schematic. The hydrophobic CF_0 appears to contain 4-6 copies of the DCCD (N,N'-dicyclohexylcarbodiimide) binding protein or subunit III but CF_0 has not yet been purified [54]. A model for isolated CF_1 has recently been proposed [75]. The subunit stoichiometry shown here is $\alpha 3\beta 3\alpha\gamma\delta\varepsilon$ [54].

Q_A is reduced. When K_I' is >> K_O' centers become stable in the state Q_A^-I. Since Q_A^- is not a quencher of fluorescence, the presence of Q_A^-I may be detected by measurments of the variable Chl *a* fluorescence yield. In the presence of DCMU, however, the formation of centers in the state Q_A^-I appears to be present only in 50-70% of PSII [48-51,92]. This apparent partial displacement of Q_B has been attributed to heterogeneity of PSII electron acceptors rather than equilibrium between the possible states indicated in Fig. 3. Centers which do exhibit electron back-transfer from Q_B^- to Q_A in the presence of DCMU are known as B-type; those accounting for the remainder of the variable fluorescence are described as non-B-type. Lavergne [50] has suggested that non-B-type centers are not connected to the main electron transfer pathway; and further B-type centers possess many characteristics of Q_1 centers while non-B-type centers resemble Q_2 centers [2].

There is an additional complexity. PSIIα and PSIIβ centers are characterized by kinetic components of the steady-state fluorescence induction curve. The Chl *a* transient, in the presence of DCMU, exhibits a fast sigmoidal phase corresponding to PSIIα and a slower exponential phase corresponding to PSIIβ [56,57]. The sigmoidicity of the α phase has been suggested to arise as a consequence of interconnected antennae serving these centers. In this matrix model [36] the α-centers exist in a statistical pigment bed (see also [8]). Energy transfer is allowed between PSIIα units such that an exciton arriving at a closed reaction center is able to visit other centers until it encounters an open trap. The first-order kinetics of PSIIβ centers, by contrast, arise from centers where energy transfer from closed to open centers is not possible. It has been proposed that PSIIα are associated with stacked appressed thylakoid membranes and PSIIβ is present in the stroma lamellae (see *e.g.*, [1]). Studies employing absorbance difference spectroscopy have shown that while α-centers contain Q_1, β-centers contain both Q_1 and Q_2 [55,58].

Recently, a population of PSII centers have been identified that are able to evolve oxygen in the presence of halogenated benzoquinones, artificial electron acceptors for PSII, but are not connected to the main electron transport pathway (T. Graan and D. R. Ort, personal communication). These centers appear to represent about 40% of the total PSII present. The relationship of these centers to other PSII heterogeneities has yet to be characterized.

For further details of PSII the reader is referred to published reviews [2,26,30,76,85].

In the remainder of this overview we shall discuss some of the studies that have explored the effects of anions on the acceptor side of

PSII. Electron transfer at the level of the two-electron gate has been shown to be inhibited by the presence of formate and NO_2^- ([15,25,62]). Whether this effect is due to the removal of bound HCO_3^- or it is a direct inhibitory effect is not yet clear. However, this inhibition is uniquely reversed by the addition of HCO_3^- (see e.g., [21,74]). Furthermore, a wide range of monovalent anions have been shown to be competitive inhibitors of HCO_3^- binding [74; cf. 21]. These findings suggest the existence of an anion binding site on PSII that, when occupied by HCO_3^-, facilitates electron transport into the PQ pool. We are currently investigating the possibility that acetate, formate and NO_2^- inhibit electron flow [74] by displacing HCO_3^-. An alternative approach is that the stimulation of electron transport by HCO_3^- is simply due to the removal of inhibitory anions [68,71].

2. THE BICARBONATE EFFECT

Warburg and Krippahl [91] reported a stimulatory effect of HCO_3^- on the Hill reaction. Originally, it was assumed by Warburg that this effect was on the oxygen evolving mechanism, i.e., he assumed that O_2 was evolved from CO_2. Recent studies have shown [23,84,87] that this effect is on the electron acceptor side of PSII.

Good [21] studied the conditions necessary for HCO_3^--depletion and found that the presence of anions, particularly formate, acetate and chloride, facilitated the depletion process. Since HCO_3^- in solution sets up the following equilibria:

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^- \rightleftarrows 2H^+ + CO_3^{2-} \qquad (1)$$

the nature of the active species involved has been the subject of several studies. The most effective pH to stimulate the Hill reaction in HCO_3^--depleted thylakoids, upon addition of HCO_3^-, was found to be in the pH 6-7 range [46,70]. In confirmation of this, the maximal HCO_3^--restored/HCO_3^--depleted ratio of Hill reaction rates was found to be at pH 6.5 [88]. Furthermore, addition of CO_2 to HCO_3^--depleted samples was found to stimulate Hill activity more readily than addition of HCO_3^- to HCO_3^--depleted samples at 5°C and pH 7.3 [64,65]. Since the pK for the overall reaction ($CO_2 + H_2O \rightleftarrows H^+ + HCO_3^-$) is 6.4, it was suggested (e.g., [88]) that CO_2 was the species required for diffusion to the active site in HCO_3^--depleted membranes but that HCO_3^- was the active species in restoring the activity. That the active species is indeed HCO_3^- has been recently shown [4,15] by taking advantage of the pH dependence of $[CO_2]/[HCO_3^-]$ ratio at equilibrium. The rate of restored electron transport, in HCO_3^--depleted membranes in the presence of formate, was found to depend on the HCO_3^- concentration when the CO_2 concentration was held constant. This work also demonstrated that H_2CO_3 and CO_3^{2-} have no direct involvement in reversing HCO_3^--depletion.

The location of the HCO_3^- effect in the electron transport chain has been identified through several approaches. Wydrzynski and Govindjee [94] studied the effect of this phenomenon on the Chl a fluorescence induction kinetics and observed an accelerated rise in HCO_3^--depleted samples. This demonstrated that the reoxidation of Q_A^- had been impaired in the depleted samples. Employing specific inhibitors and electron donors and acceptors, which enabled the electron transport chain to be dissected into a number of clearly defined partial reactions, the HCO_3^- effect was located on the electron acceptor side of PSII [14,46]. Competitive binding studies with

several PSII herbicides, which bind near Q_B, also support this view [47, 67,73,79,80,89]. We anticipate that a study of the HCO_3^- specific reversal of anionic inhibition will add substantially to our understanding of PSII acceptor side chemistry.

3. ANIONIC INTERACTIONS ON PSII ACCEPTOR SIDE QUINONE CHEMISTRY

Kinetics of Q_A^- reoxidation may be followed by monitoring the decay of Chl a variable fluorescence by a double-flash technique [53]. Following a single-turnover actinic flash, a second weak flash, sampling approximately 1% of the centers [35], is given at specified times. The fluorescence yield from the weak analytical flash is a function of $[Q_A^-]$, the relationship being non-linear [18,36]. Adoption of this technique has shown Q_A^- reoxidation to be inhibited identically in samples HCO_3^--depleted in the presence of formate [25,41,43,62], and similar samples even in the presence of atmospheric CO_2 (390 µl/l) [62]. This phenomenon has also been measured by the absorbance change at 320 nm [17,66] and by the 515 nm absorbance change both in thylakoids [41] and in intact chloroplasts [78]. No specific measurements have been made yet to address the differential effects, if any, of this inhibition upon the various PSII heterogenous populations. However, it is evident from the correlation between the fluorescence and absorption measurements that this phenomenon is associated with Q_1 i.e., the HCO_3^- effect is in the major PSII centers.

The extent of the anionic interaction is dependent upon flash number [25]. Using Chl a fluorescence, we have measured [62] half-times for Q_A^- reoxidation of 1.2 ms for HCO_3^--depleted and formate incubated samples, and 230 µs for control and HCO_3^--restored thylakoids after a single flash. After the third flash, we obtained half-times of 13 ms for HCO_3^--depleted, 10 ms for formate-incubated, and 360 µs for control and HCO_3^--restored samples. The half-times after flash 2 were intermediate between flash 1 and 3; subsequent flashes yielded results similar to flash 3. The above conclusion was also evident from absorbance changes at 320 nm [17] and 515 nm [78].

The kinetics of Q_A^- reoxidation for flash 3 are expected to resemble those of flash 1 [60] since, following the formation of plastoquinol after the second flash, $Q_B^{2-}(2H^+)$ should readily exchange with a PQ from the PQ pool and Q_A^- should be oxidized by this PQ species. The exchange reactions at the B-site have been determined to occur with a half-time < 2.5 ms (Robinson, H.H. and Crofts, A.R., personal communication). We have found [15] that the inhibition for the third flash in HCO_3^--depleted/anion inhibited centers is large and is the same when the dark time between the second and third flashes is 30 ms or 1 s; this result indicates that the exchange reactions are greatly decreased in the inhibited or HCO_3^--depleted case.

A possible explanation for the above observations is that the binding of inhibitory anions to PSII may alter the association constant, K_Q for Q_B (see Fig. 3). Although there is no direct measure of the value for K_Q, a number of methods for estimating a value are available [10]. One method is to analyze the decay kinetics of Q_A^- by monitoring the variable Chl a fluorescence after a single flash. Biphasic kinetics are observed for this decay; 60-70% of centers undergo oxidation by a first-order process with a half-time of ~0.15 ms and the remainder by slower processes of indeterminate order [10]. If it is assumed that the centers exhibiting first-order kinetics represent centers in the state Q_AQ_B before the flash,

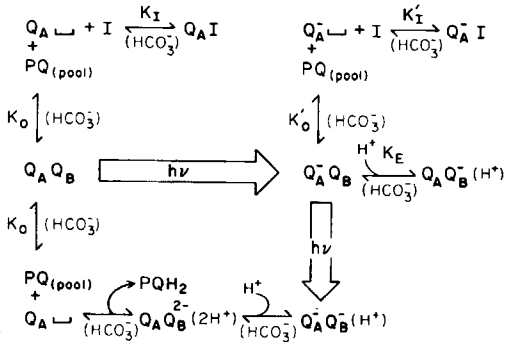

FIGURE 3. Diagramatic presentation of the possible reactions associated with the secondary quinone binding site of the B-site. Photochemical reactions are shown as open arrows; ⊥_____⊥ represents the empty B-site. K_o and K_I are the association constants for plastoquinone and herbicide respectively when Q_A is oxidized and K'_o and K'_I are the association constants when Q_A is reduced. K_E is the equilibrium constant for the sharing of an electron between Q_A and Q_B. The reactions apparently influenced by HCO_3^- are indicated (see [15] and text for details).

a value of 500 M^{-1} for K_o can be calculated [10]. We have analyzed our earlier data for HCO_3^--depleted and formate-incubated thylakoids [62] and found that K_o is reduced to 200 M^{-1} in these samples [15]. A second effect is also evident from this analysis. The half-time of the fast phase is increased approximately 4-fold (i.e., from ~0.2 ms to ~0.8 ms) in these samples [cf. 62]. The mechanism of this second effect cannot be explained from the available data.

In addition to the slowing and reduction of the fast phase of Q_A^- reoxidation, a shift in the equilibrium for the sharing of an electron between Q_A and Q_B (see Fig. 3, K_E) has been reported in the thylakoids that have been HCO_3^--depleted in the presence of formate [90]. A two-fold shift in this equilibrium towards Q_A^- was observed by comparing the rates of the back-reaction with the S_2 state (for a discussion of S-states, see [26]) of the oxygen evolving complex both in the presence and absence of DCMU. In the absence of DCMU, the back-reaction from Q_B^- to S_2 was inhibited two-fold [90].

The equilibrium (see Fig. 3, K_E) for the sharing of an electron between Q_A and Q_B is pH dependent [61]. It has, therefore, been suggested that the presence of a proton in association with the B-site stabilizes the electron on $Q_B^-(H^+)$. We suggest that HCO_3^--depletion inhibits protonation at the B-site. In addition, the fraction of centers decaying through the rapid first-order process after a second flash, has been shown to be proportional to the fraction of centers in which $Q_B^-(H^+)$ is present [61]. Therefore the inhibition on the $Q_B^-(H^+)$ protonation suggested above may also account for the inhibited kinetics of Q_A^- reoxidation observed after the second flash [25,62]. By analogy, the maximal inhibition observed after the third flash may result from Q_B^{2-} not becoming protonated and therefore not able to exchange with the PQ pool. This interpretation suggests that the rate-limiting step introduced by HCO_3^--depletion and/or anion

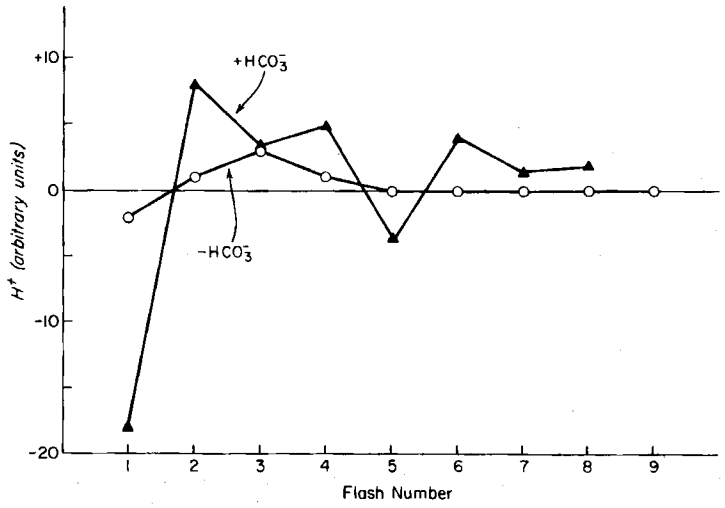

FIGURE 4. The effect of HCO_3^--depletion in the presence of formate on the proton oscillations in uncoupled pea thylakoids measured by a sensitive pH electrode. The electron acceptor was methyl viologen; the sample included methylamine and gramicidin-D to allow the protons to leak out of the thylakoids (from [27]).

inhibition is the rate of protonation of Q_B^{2-}. A role for HCO_3^- in protolytic reactions in PSII has also been proposed as a result of comparative studies with carbonic anhydrase [69,71].

Although an effect of HCO_3^- on protonation at the B-site has not been shown, an effect of HCO_3^--depletion, in the presence of formate, has been shown by Govindjee et al. [27] on proton release at the level of PQH_2 oxidation. Using ferricyanide or methyl viologen as electron acceptor, and an uncoupler of phosphorylation (e.g., methylamine) to bring the protons released into the lumen into the vicinity of a sensitive pH electrode, Fowler [20] had measured an oscillation with a combined period of 4 (protons released from the oxygen evolving complex) and of 2 (protons released from PQH_2 oxidation). This was confirmed by Govindjee et al. [27] for the first time by a pH electrode, although confirmation and extension by other methods have already been made (see e.g., Förster and Junge [19]). When the samples were depleted of HCO_3^-, the binary oscillation was abolished (Fig. 4). This result is consistent with our picture that HCO_3^--depletion blocks electron flow prior to PQH_2 oxidation. Unfortunately, this result does not provide any clue as to any direct effect of anions on the protonation reactions per se.

4. THE BINDING CONSTANT FOR BICARBONATE AT THE ANION SITE

Given the unique ability of HCO_3^- to reverse the anion inhibition of quinone mediated acceptor side electron transfer, studies have been performed to determine its binding constant (K_b). A value of 80 μM has recently been obtained using $H^{14}CO_3^-$ in maize thylakoids with 1 binding site per PSII [72,73]. $H^{14}CO_3^-$ binding has been shown to be competitive

with HCO_2^-, NO_2^-, NO_3^-, and $CH_3CO_2^-$ and F^- [74]. This list is almost certainly not exhaustive. NO_2^- is of particular interest. Formate has routinely been employed in HCO_3^--depletion procedures since the work of Good [21]. This reflects, in part, its structural homology with HCO_3^- as well as its specificity. NO_2^-, however, has the same degree of charge delocalization as does HCO_3^-. Blubaugh and Govindjee [4] have discussed the significance of this homology suggesting that the unique behavior of HCO_3^- may result from the hydroxyl group on this anion. A similar suggestion was made earlier by Good [21]. Stemler and Murphy [74] have demonstrated that NO_2^- is an even more effective competitor of $H^{14}CO_3^-$-binding than formate. We have reported [15] that HCO_3^--depletion can also inhibit Q_A^- reoxidation and steady-state electron transport supported by methyl viologen when formate is replaced by NO_2^- in HCO_3^--depletion and reaction media. Jursinic and Stemler [42] have also demonstrated, using identical experimental conditions as employed for the $H^{14}CO_3^-$-binding constant determination, an 80 μM K_m (concentration required to restore half-maximal activity) for Q_A^- reoxidation as monitored by the decay of variable Chl *a* fluorescence following a single actinic flash. These findings therefore appear to confirm that the binding constant measured in $H^{14}CO_3^-$-binding studies is for the binding site at which HCO_3^- facilitates electron transfer through PSII in the presence of inhibitory anions.

However, the magnitude of the HCO_3^--binding constant may have been over-estimated. Since it has been shown that HCO_3^- and various anions are competitive at the 80 μM site [74], it follows *a priori* that the K_b for HCO_3^- will depend upon the anionic strength used in the experimental conditions. The K_b determination was in fact performed in buffers containing 200 mM NaCl, which is much higher than the $[Cl^-]$ needed for PSII activity [33]. This level of Cl^- has already been established to facilitate HCO_3^--depletion almost certainly by increasing the binding constant. In fact, we have demonstrated [15] that the time course of HCO_3^-depletion is dependent on the $[Cl^-]$. Thus, the binding constant under native conditions is expected to be smaller than 80 μM.

Furthermore, it is difficult, if not impossible, to be sure that there is only one binding site and only one binding constant. A hint of at least two separate binding sites was presented by Blubaugh and Govindjee [3]. The existence of a tight binding site may have been overlooked since none of the experiments show data on the amount of intrinsic bound HCO_3^- in the sample.

5. MECHANISM OF BICARBONATE ACTION AND POSSIBLE PHYSIOLOGICAL ROLES FOR BICARBONATE

The specificity of HCO_3^- in reversing the inhibition induced by anions on PSII acceptors has lead to speculation regarding an *in vivo* role for HCO_3^-. The phenomenon is clearly associated with PSII-Q_1-B-type centers and therefore is a characteristic of the principal electron transport pathway. Bound HCO_3^- has also been suggested [47] to produce a conformational change in the 32 kD herbicide/quinone binding protein (Fig. 2), facilitating efficient reduction of Q_B [86], and of exchange of $Q_B^{2-}(2H^+)$ with a PQ molecule of the PQ pool (Fig. 3). Indeed, the phenomenon is irrefutably associated with the oxidation of Q_A^- in these centers and strong evidence suggesting a direct involvement on the exchange reactions of the two-electron gate has been collected [15,25,29,62]. Herbicide action has even been proposed to result from the displacement of HCO_3^- from its binding site [80,88].

One physiological role suggested is that HCO_3^- may act as a regulatory anion balancing the production of ATP and reductant (NADPH) needed for CO_2 assimilation [84]. A detailed scheme has been proposed where HCO_3^- protects against inhibitory formate produced in photorespiration [68]. A HCO_3^- effect has been shown in the Hill reaction by intact chloroplasts [78] and by intact cells in the presence of formate. Figure 5 shows measurements of Govindjee et al. [28] on HCO_3^- reversible anionic inhibition of O_2 evolution in intact cells of a green alga Scenedesmus; in these experiments, HCO_3^--depletion of cells was done by first letting the cells perform photosynthesis and use up ambient CO_2, then formate was added to remove bound HCO_3^-, and parabenzoquinone was used to diminish respiration. Furthermore, the parabenzoquinone Hill reaction was measured in order to separate the HCO_3^- effect from that due to the operation of CO_2 fixation. It is clear from Fig. 5 that HCO_3^- was required for the Hill reaction by Scenedesmus cells. The insert in Fig. 5 shows the Chl a fluorescence transient of Scenedesmus cells without the addition of parabenzoquinone. The results, shown here, are similar to those on chloroplasts [86]. Apparently, this suggests that the two have the same basis. This is further supported by the data on other green algae in which electron acceptors beyond ferredoxin-NADP reductase had no effect on the fluorescence transient (see discussions by Govindjee and Satoh [22]). However, we cannot reject the possibility that the absence of CO_2 fixation in CO_2-free cells may also give a faster rising fluorescence transient.

Attempts to see the HCO_3^- effect in the absence of inhibitory anions have met with partial success. These results are reminescent of the early days of HCO_3^- research when small HCO_3^- effects on the Hill reaction were observed without the use of inhibitory anions [23,91] and large effects with inhibitory anions present [21,23]. In the absence of inhibitory anions, we have observed a fully reversible HCO_3^- effect on Q_A^- reoxidation [14]; this effect is also present in steady-state oxygen evolution and in the Chl a fluorescence induction kinetics [15]. The effect, however, is less dramatic than when inhibitory anions are present. For example, after 3 actinic flashes the kinetics of Q_A^- reoxidation for HCO_3^--depleted thylakoids were found to have a half-time of approximately 2 ms in the absence of formate [14]. This is to be compared with approximately 13 ms in the presence of formate [62]. However, 20 mM Cl^- was present in the formate free case. It is possible that in the absence of HCO_3^- this low [Cl^-] might become inhibitory. Bound HCO_3^- may possibly be necessary in vivo to protect against inhibition from anions such as $CH_3CO_2^-$, NO_2^- and Cl^- as suggested for formate [68].

Arguments against a physiological role for HCO_3^- have been based upon the 80 μM binding constant [72]. This claim stems from an estimated in vivo CO_2 concentration of < 5 μM [32] which, it has been suggested, would result in the HCO_3^- binding site being unoccupied. However, Blubaugh and Govindjee [4] have pointed out that while the CO_2 concentration may be quite low in the chloroplast, at pH 8.0, the approximate pH of the stroma, the HCO_3^- concentration may be as high as 220 μM. This is well above the estimated binding constant [4,15]. Furthermore, the binding constant under native conditions may even be much lower than 80 μM.

The last word of this debate has not yet been heard. However HCO_3^- reversible anionic inhibition of PSII is clearly a real phenomenon and has already proved itself an important gateway into the complex reactions of the acceptor side of PSII.

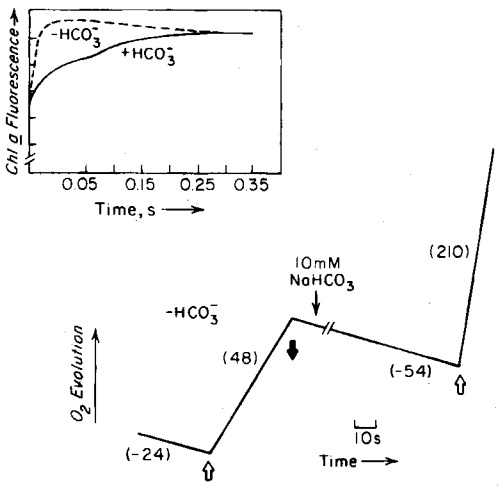

FIGURE 5. The effect of HCO_3^--depletion in the presence of formate on the Hill reaction in intact cells of Scenedesmus. The electron acceptor was parabenzoquinone. The numbers in parentheses indicate O_2 exchange in μmoles(mg Chl)$^{-1}$ h^{-1}. Open and closed arrows indicate light on, and off, respectively. The insert shows the effect of HCO_3^--depletion, in the presence of formate but in the absence of parabenzoquinone, on the chlorophyll a fluorescence transient (from [28]).

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ADDENDUM

W.F.J. Vermaas and A.W. Rutherford (FEBS Lett 175: 243-247, 1984) have reported that the EPR signal of the iron-quinone in PSII is much larger in HCO_3^--depleted particles. This suggests an interaction of HCO_3^- at the Q_A-Q_B level. M.C.W. Evans (Physiol Veg 23: 563-569, 1985) has looked at this iron-quinone in HCO_3^--depleted PSII samples by EPR and observed two signals with E_m = 50 mV and E_m = -250 mV. On the basis of this and other results Evans has presented a model for electron flow from Q_A to PQ which is different from that presented in this overview. In the Evans' model, two bound semiquinones act in a concerted fashion to reduce a PQ molecule. Further experiments are needed to judge the merits of this proposal.

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