Bicarbonate or Carbon Dioxide as a Requirement for Efficient Electron Transport on the Acceptor Side of Photosystem II

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ABBREVIATIONS

B (or R) Second quinone-type PSII electron acceptor
Chl Chlorophyll
DAD 2,3,5,6-Tetramethylphenylenediamine
DBMIB 2,5-Dibromo-3-methyl-6-isopropyl-p-benzoquinone
DCMU 3-(3,4-Dichlorophenyl)-1,1-dimethylurea
DCPIP 2,6-Dichlorophenolindophenol
DPC Diphenylcarbazide
EPR Electron paramagnetic resonance
FeCy Ferricyanide [Fe(CN)$_6^{3-}$]
HCO$_3^-$ Species (CO$_2$ or HCO$_3^-$) that binds to a specific binding site and allows efficient electron transport ($\equiv$ CO$_2^\delta$ in Stepler, Chapter 15)

*This chapter should be read in conjunction with the chapter by A. Stepler—Editor.
ABSTRACT

In this chapter, we reviewed observations showing that HCO$_3^-$ (CO$_2$ or HCO$_3^-$) greatly affects the electron transport rates between the quinone-type intermediates (Q, B, and the PQ (plastoquinone) pool) on the acceptor side of photosystem II (PSII) without directly influencing the donor side of PSII. The major observations against an important role of HCO$_3^-$ on the donor (oxidizing) side and in favor of its role on the acceptor (reducing) side of PSII are

1. There is no HCO$_3^-$ effect on the electron transport from H$_2$O to Q as measured with siliconolybdate as electron acceptor in the presence of DCMU or with ferri-cyanide in trypsin-treated chloroplasts (Section II,A).
2. There is no HCO$_3^-$ effect on the kinetics of electron flow from the oxygen evolving system to the electron donor Z to P680, and from Z to P680 (Section II,E).
3. There is a large HCO$_3^-$ effect on electron transport from Q$^-$ to B, as measured by both fluorescence and absorption methods (Sections II,B, and II,C); an additional, but smaller, effect is observed between P680 and Q.
4. The Chl a fluorescence transient in CO$_2$-depleted samples is similar, but not identical, to that in the presence of DCMU; the latter blocks electron flow beyond Q. Detailed analysis suggests that the absence of HCO$_3^-$ causes a major block after B (Section II). A large effect on the electron transport from B$_2^-$ to PQ is also confirmed by measurements of (a) Chl a fluorescence after single flashes of light; and (b) absorption changes at 265 nm (Sections II,B and C).
5. The location of the binding site of HCO$_3^-$ is close to that of the herbicides that bind near Q and B (Section II,F).

The bottle-neck reaction in CO$_2$-depleted chloroplasts seems to be electron transport from B$_2^-$ to PQ, with a $t_{1/2}$ of at least 100 msec; in thoroughly depleted samples, this reaction takes seconds suggesting a "complete" block. After HCO$_3^-$ addition, the PQH$_2$ oxidation ($t_{1/2} = 25$ msec) becomes rate-limiting, like in control chloroplasts. Most of the Q$^-$ reoxidation by B$^-$ is faster than the PQ reduction in CO$_2$-depleted chloroplasts. No experimental data, thus far, point unequivocally to any CO$_2$/HCO$_3^-$ effect on the donor side of PSII.

This chapter does not offer a complete picture of the HCO$_3^-$ or CO$_2$ action in thylakoid membranes; it only deals with the site of action of the "bicarbonate effect" on photosynthetic electron transport. Therefore, in order to obtain an "overview" of this subject, this chapter should be read in conjunction with the cited reviews and with Chapter 15.

†In this chapter, the description "B$_2^-$" is used to denote fully reduced B in general, and the use of "B$_2^-$" does not exclude protonation of the reduced form of this secondary quinone.
I. Introduction

A crucial role of CO₂ or bicarbonate on the oxygen evolving mechanism of photosynthesis, as presented in Chapter 15 of this volume, is a point of view that is interesting but speculative inasmuch as it does not yet have a firm experimental backing. The present chapter is written to provide the reader with information on the action of CO₂ or HCO₃⁻ on the acceptor side of PSII, for which, contrary to a bicarbonate effect on the donor side of PSII, strong evidence exists. The absence of HCO₃⁻ results in a severe slowing down of electron transport between Q, the first quinone-type PSII acceptor, and the PQ pool, without directly affecting electron transport on the donor side of PSII (see Fig. 1). This effect of HCO₃⁻ has been established by many types of experiments in several laboratories (see, e.g., Wydzynski and Govindjee, 1975; Govindjee et al., 1976; Jursinic et al., 1976; Khanna et al., 1977, 1980, 1981; Siggel et al., 1977; Stemler, 1977, 1979; van Rensen and Vermaas, 1981a; Vermaas and van Rensen, 1981). Some of these experiments will be summarized later. It is our hope that the reader after having read this and Chapter 15 may have a better appreciation of the complicated, but interesting and important, bicarbonate problem.

In addition to its effect on electron transport, bicarbonate has been shown to influence other photosynthetic processes in the thylakoid membrane, including enhancement of photophosphorylation at pH 7.0–7.5 (Punnett and Iyer, 1964). However, at pH 8.0, the pH optimum for photophosphorylation, no stimulation of phosphorylation is observed. Nelson et al. (1972) concluded from their experiments with isolated coupling factor protein that HCO₃⁻ might cause a conformational change in this protein. This suggestion has been confirmed by Cohen and MacPeek (1980).

In this chapter, we will concentrate on the HCO₃⁻ effects on photosynthetic electron transport. For a background on electron transport, the reader is referred to Vermaas and Govindjee (1981b) and Crämer and Crofts, Chapter 9, Vol. I (1982). No attempt is made to speculate on the “active species,” i.e., the species (CO₂, HCO₃⁻ or CO₃⁻) that is responsible for restoration of electron transport or about the molecular mechanism of the specific HCO₃⁻ binding to the thylakoid membrane. For publications that cover these questions, see Govindjee and van Rensen (1978), Stemler (Chapter 15, this volume), Vermaas and Govindjee

*Since it is unknown whether CO₂ or HCO₃⁻ is necessary for efficient electron transport, we will use the description HCO₃⁻ to denote the CO₂ or HCO₃⁻ that is specifically bound to the membrane and that exerts its effect on electron transport.
FIG. 1. Pathway of noncyclic electron flow from H₂O, the electron donor in photosynthesis, to NADP⁺ (nicotinamide adenine dinucleotide phosphate), the "physiological" electron acceptor. E on the ordinate stands for midpoint redox potential. Light quanta (hv) are absorbed in two sets of antenna Chl molecules; the excitation energy is transferred to the reaction center Chl a molecules of PSII (P680) and of PSI (P700), which forms (P680)⁺ and (P700)⁺, respectively. The latter two initiate electron transport. Inhibitors of photosynthetic electron transport are known to work at specific sites in the electron transport chain. Inhibition of electron transport by a certain treatment or molecule is indicated as →, slowing down of electron transport as ←, and electron donation or acceptance by an artificial donor or acceptor is drawn as a curved line. M₀⁻⁴, the charge accumulator of the oxygen-evolving system that can exist in several charged states; D, an unknown electron donor to P680; FeS, the Rieske iron–sulfur center; cyt f, cytochrome f; PC, plastocyanin; A₁, the primary PSI acceptor, is suggested to be a Chl molecule; A₂, an iron containing protein, which is considered equivalent to the so-called X; (A → B) center, iron–sulfur centers observed in EPR spectra; and Fd, ferredoxin. (Also see the List of Abbreviations.) (For further details, see Volume I, also edited by Govindjee, 1982.)


II. A Site of HCO₃⁻⁺ Action on the Acceptor Side but Not on the Donor Side of Photosystem II

Warburg and Krippahl (1958, 1960) showed that HCO₃⁻⁺ is necessary for efficient electron transport in thylakoids or intact Chlorella cells,
using quinone as an artificial electron acceptor. After this discovery, several groups investigated this “bicarbonate effect” further (see, e.g., Abeles et al., 1961; Stern and Vennesland, 1962; Good, 1963; West and Hill, 1967). These initial studies were limited due to variability in the CO$_2$-depleted material. Once a reproducible method for CO$_2$ depletion was developed (Stemler and Govindjee, 1973), a determination of the site of HCO$_3^-$* action was possible. Although at first, Stemler and Govindjee (1973) observed no bicarbonate effect (i.e., stimulation of electron transport by HCO$_3^-$ addition) using DPC (diphenylcarbazide) and DCPIP (2,6-dichlorophenolindophenol) as artificial donor and acceptor, respectively, experiments performed later by Wydrzynski and Govindjee (1975) showed a significant bicarbonate effect on the DPC $\rightarrow$ DCPIP reaction, but the effect was lower than in the H$_2$O to DCPIP Hill reaction. These observations could indicate an action of HCO$_3^-$* both on the acceptor and on the donor side of PSII. However, care should be taken in interpreting these data because DPC might have effects on the electron transport chain other than electron donation to the donor Z of the PSII reaction center (P680), e.g., an increase in the efficiency of PSII (Harnischfeger, 1974). Fischer and Metzner (1981) showed that the methyl viologen-mediated Mehler reaction (monitoring noncyclic electron transfer through PSII and PSI) in thylakoids treated with hydroxylamine (blocking P680$^+$ reduction by the physiological donor Z and reducing P680$^+$ via an unknown donor D) is relatively insensitive to HCO$_3^-$*. However, Wydrzynski and Govindjee (1975) observed a large HCO$_3^-$* effect using NH$_2$OH as an electron donor. In order to resolve this uncertainty, the induction kinetics of the variable Chl a fluorescence after CO$_2$ depletion may be examined. The variable Chl a fluorescence monitors, among other things, the redox state of Q; Q is a quencher of Chl a fluorescence, whereas Q$^-$ is not (see, e.g., a review by Lavorel and Etienne, 1977). Therefore, a rapid accumulation of Q$^-$ due to an inhibition or slowing down of electron transport beyond Q is easily detected by fluorescence measurements. The CO$_2$ depletion causes a fast increase in the variable fluorescence yield (Stemler and Govindjee, 1974; Wydrzynski and Govindjee, 1975), similar to that observed in the presence of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea] (Wydrzynski and Govindjee, 1975); the latter blocks reoxidation of Q$^-$ by B($^-\),$ the second quinone-type PSII acceptor. Blockage of the donor site by heat treatment (Katoh and San Pietro, 1967; Homann, 1968) causes a much lower yield of the variable fluorescence (Wydrzynski and Govindjee, 1975). Since fluorescence induction traces of CO$_2$-depleted chloroplasts resemble, on a short time scale, traces of normal chloroplasts in the presence of DCMU, but do not resemble traces in which the donor side is blocked, it may be concluded that the primary site of HCO$_3^-$* action is
not on the oxygen evolving system (Wydrzynski and Govindjee, 1975). Furthermore, after alkaline Tris-washing (which deactivated the oxygen-evolving system (Yamashita and Butler, 1968)) and CO₂ depletion of chloroplasts, the addition of artificial PSII electron donors (e.g., DPC and NH₂OH) is unable to change \(-\text{HCO}_3^-\) fluorescence characteristics into \(+\text{HCO}_3^-\) characteristics, which points to a bicarbonate effect between the site of donation by these external donors and PQ (Wydrzynski and Govindjee, 1975). If thoroughly CO₂-depleted chloroplasts are used, a major fast rise without any significant slow phase in the fluorescence transient is observed. This points to a (nearly) complete blockage of electron transport on the acceptor side of PSII (Vermaas and Govindjee, 1982). Since the area over the fluorescence induction curve in CO₂-depleted chloroplasts is about twice as large without DCMU than with it a major block must occur beyond B (Vermaas and Govindjee, 1982). If the CO₂ depletion is not complete, then a fast rise in the fluorescence induction curve followed by a slow rise to the maximum fluorescence level is observed (Stemler and Govindjee, 1974). (For a detailed explanation of the latter data, see Vermaas and Govindjee, 1981a.)

A. Electron Transport Rates in the Presence of Artificial Electron Acceptors

In the presence of SiMo (silicomolybdate; SiMo\(_{12}\)O\(_{40}\)\(^{4-}\)) and DCMU, no significant bicarbonate effect is observed in CO₂-depleted chloroplasts (Khanna et al., 1977; van Rensen and Vermaas, 1981a). [SiMo is an artificial acceptor known to accept electrons from Q (Giaquinta and Dilley, 1975; Zilinskas and Govindjee, 1975).] It is difficult to imagine a significant CO₂-depletion effect on the donor side of PSII that would not inhibit electron flow from H\(_2\)O through P680 to SiMo. However, the addition of HCO\(_3^-\) causes a dramatic increase in the Hill reaction with oxidized DAD (2,3,5,6-tetramethylphenylenediamine) as acceptor when DBMIB (2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone) is present to inhibit electron flow beyond the PQ pool (Khanna et al., 1977; Sarojini et al., 1981; see also Fig. 1). No measurable effect of HCO\(_3^-\) is observed in a PSI reaction as monitored by electron flow from reduced DAD to MV (methyl viologen) in the presence of DCMU (Khanna et al., 1977). These data indicate that the most important effect of HCO\(_3^-\) is between Q and PQ and not on the donor side of PSII, since the reaction H\(_2\)O → SiMo appears to be almost insensitive to 10 mM bicarbonate. Further support against an effect of HCO\(_3^-\) on the donor side of PSII is provided by data on trypsin-treated chloroplasts. Ferricyanide (FeCy) is known to be able to accept electrons directly from Q after trypsin treat-
ment (Renger, 1976; van Rensen and Kramer, 1979); after trypsin incubation, no bicarbonate effect on the FeCy Hill reaction is observed (Khanna et al., 1981; van Rensen and Vermaas, 1981b), suggesting that HCO$_3^-$* does not influence the donor side of PSII.

B. Absorption Changes Due to Quinones

An independent although somewhat more complicated way to monitor electron transport is by spectrophotometry. Flash-induced absorption changes in the 320–334-nm region are ascribed to the oxidation or reduction of Q (Stiehl and Witt, 1968; Witt, 1973; van Gorkom, 1974; Renger, 1976; Siggel et al., 1977), whereas changes in absorption at 265 nm are correlated with changes mainly in the B and PQ pool (Stiehl and Witt, 1969; Haehnel, 1976; Siggel et al., 1977).

Using repetitive flashes (with 250 msec dark time between the flashes), a part of the Q$^-$ decay, as measured by 334-nm absorbance changes, is slowed down significantly in CO$_2$-depleted chloroplasts [450 µsec (in control) → 6 msec (−HCO$_3^-$*)]. After HCO$_3^-$ addition, this slow phase nearly disappears, and the kinetics become similar to that in the control. The slowing down of the Q$^-$ decay in the absence of HCO$_3^-$* suggests a decrease in the rate of Q$^-$ oxidation by B. Furthermore, the amplitude of the absorbance change at 334 nm is smaller before than after HCO$_3^-$ addition (Siggel et al., 1977). This was interpreted as a (reversible) inactivation of P680 (Siggel et al., 1977). However, no bicarbonate effect on steady state electron transport from H$_2$O to Q has been detected (Khanna et al., 1977; van Rensen and Vermaas, 1981a); so, the explanation of a reversible P680 inactivation by CO$_2$ depletion is not supported by other data. A more reasonable explanation for this phenomenon is that part of Q$^-$ is reoxidized very slowly in the absence of HCO$_3^-$* (∼ 1 sec) (Jursinic and Stemler, 1981). Therefore, part of Q$^-$ formed after one flash is not yet reoxidized when the next flash arrives at the sample; this results in a lower [Q] just before a flash and in a lower amplitude of the absorbance change at 334 nm in the absence of HCO$_3^-$*. It is not yet known if this very slow component of Q$^-$ reoxidation is due to electron transfer to B(−) or to another electron acceptor. This means that certain PSII reaction centers are not completely “turned off” by CO$_2$ depletion, but rather have had no time to recover between flashes. Thus, absence of HCO$_3^-$* does not decrease the number of active reaction centers (P680).

When absorption changes at 265 nm that are induced by 85 msec flashes (spaced 5 sec apart) are compared in CO$_2$-depleted chloroplasts with and without HCO$_3^-$* addition, it is observed that CO$_2$ depletion
reduces the amplitude of the 265 nm signal and slows down its decay. The kinetics of formation of the signal are determined by the opening time of the shutter (≈ 10 msec) and are not significantly influenced by HCO$_3^-$*. To explain these data, it was suggested that in the absence of HCO$_3^-$ the reduction of the PQ pool is slow compared to its oxidation, causing a low steady state [PQH$_2$] during illumination and, therefore, a small absorbance change. The observed absorbance change was interpreted to be due mainly to the formation of B$^{2-}$.$^+$ Then, the slow decay has to be ascribed to a slow electron transport from B$^{2-}$ to PQ ($t_{1/2}$ ≈ 100 msec), followed by a relatively fast reoxidation of PQH$_2$ ($t_{1/2}$ ≈ 25 msec) (Siggel et al., 1977). Further experiments are needed to confirm these interpretations.

C. Chlorophyll a Fluorescence Yields after Light Flashes

Experiments pointing to a significant slowing down of the oxidation of B$^{2-}$ by PQ in CO$_2$-depleted chloroplasts were presented by Govindjee et al. (1976). When CO$_2$-depleted chloroplasts are subjected to one or two saturating flashes, spaced approximately 30 msec apart, the Chl a fluorescence yield 160 msec after the flash(es) are as low as in CO$_2$-depleted chloroplasts after HCO$_3^-$ addition (Fig. 2). This observation indicates a reasonably fast (< 30 msec) reoxidation of Q$^-$ after the first two flashes in CO$_2$-depleted chloroplasts. However, when three or more flashes are applied the fluorescence yield (and, thus, the Q$^-$ concentration) 160 msec after the last flash is much higher in these chloroplasts than in CO$_2$-depleted chloroplasts to which HCO$_3^-$ is added (Fig. 2). These data show a relatively efficient electron transport from Q$^-$ to B$^{(-)}$ of only two electrons in CO$_2$-depleted chloroplasts. This is the number of electrons that can be accepted by B after dark adaptation when there is no appreciable electron transport from B to PQ. Although the dark time between the flashes in these experiments was somewhat short (not all Q$^-$ after the first flashes is oxidized before the next flash reaches the sample; see Jursinic and Stemler, 1981), these data strongly imply the existence of the main site of HCO$_3^-$* action on the oxidation of B$^{2-}$ instead of on the oxidation of Q$^-$. If the oxidation of Q$^-$ were to be slowed down by CO$_2$ depletion into the 100 msec range, the fluorescence 160 msec after the first two flashes would be as high as after the other flashes. This was not observed (Fig. 2). Therefore, these data point to a lack of reoxidation of B$^{2-}$ and, thus, to a severe slowing down of electron transport from B to PQ. However, quantitative analyses require further experiments.

$^+$See footnote on p. 542.
The preceding conclusion is confirmed by experiments in which DCMU is added to a sample after a certain number of flashes. DCMU addition is known to shift the $Q^{-}B \rightleftharpoons QB^{-}$ equilibrium to the left (Velthuys and Amesz, 1974) and is a useful probe to monitor the redox state of B by means of fluorescence, which is sensitive to the redox state of Q. In CO$_2$-depleted chloroplasts to which HCO$_3^-$ is added, a damped oscillation of fluorescence as a function of flash number (with a period of 2) is observed when DCMU is added before fluorescence is measured (Fig. 3). This observation is expected if electron transport between B$^2-$ and PQ is relatively fast. B is mainly in the oxidized form after dark adaptation, and DCMU addition does not cause a large back reaction (i.e., oxidation of B$^-$ or, perhaps, B$^2-$ by Q) because [B$^-$] is low and [B$^2-$] negligible. After one flash, B is mainly in the B$^-$ form just before DCMU is added (B$^-$ is relatively stable), resulting in a high fluorescence yield. After two flashes, B is mainly fully oxidized (the B$^2-$ oxidation by PQ is much faster than the DCMU addition). The third and fourth flashes give results similar to the first and second flashes, respectively. However, in CO$_2$-depleted chloroplasts such an oscillation with a period of 2 is not observed (Fig. 3); after dark adaptation the fluorescence yield is low, whereas after one flash a high fluorescence intensity is observed. The fluorescence yield after DCMU addition remains high after the subsequent flashes, pointing to a high relative concentration of B$^-$ and/or B$^2-$ in these samples after one or more flashes. A reasonable explanation for this observation is to assume that most of the reduced B formed by a flash is still present when DCMU is added. This indicates a prolonged lifetime of reduced B in CO$_2$-depleted chloroplasts (Govindjee et al., 1976).
D. Light-Induced Proton Uptake and Release by the Thylakoid Membrane

Proton uptake and release by thylakoid membranes can be monitored spectrophotometrically using indicator dyes and appropriate buffers in an accurate and elegant manner (Junge and Ausländer, 1973; Junge et al., 1979). When flash-induced pH changes in the internal space of the thylakoid are measured in CO$_2$-depleted chloroplasts, the amplitude of the rapid pH change due to proton production by the oxygen-evolving system is decreased compared to control chloroplasts, while a slow component ($t_{1/2} \sim 100$ msec), present in control chloroplasts, disappears (Khanna et al., 1980). This slow component is related to the oxidation of PQH$_2$, which causes proton movement into the inside (Ausländer and Junge, 1975). The absence of this component indicates that PQH$_2$ is formed only very slowly in CO$_2$-depleted chloroplasts (Khanna et al., 1980). The decrease in amplitude of the rapid phase (due to H$^+$ release into the intrathylakoid space) was interpreted by Khanna et al. (1980) as indicating an inactivation of PSII. This is probably due to an irreversible inactivation by the depletion procedure, and not to a reversible inactivation by a HCO$_3^-$ effect on the Q$^-$ oxidation as the dark time between the flashes (10 sec) was long enough to obtain full recovery of Q. Khanna et al. (1980) could not determine the reversibility of these effects because readdition of HCO$_3^-$ caused an increase in the buffering capacity of the
system, resulting in a dramatic decrease of the pH changes. Further experiments are needed to test the reversibility of these effects. Our tentative interpretation is that CO₂ depletion affects the PQH₂ formation much more than the O₂ evolution. (The changes in the PQH₂ formation rate are expected to be reversible by HCO₃⁻ addition, whereas the change in O₂ evolution is probably an irreversible “artifact” of the depletion procedure.)

E. Reduction of the Electron Donor Z and of the Photosystem II Reaction Center

To investigate if HCO₃⁻* causes changes in P680⁺ and Z⁺ reduction kinetics, which would be expected if HCO₃⁻* had any effect on the donor side of PSII, fluorescence measurements in the µsec range were performed, and the decay kinetics of the EPR signal Πᵥᵢ were recorded (Jurisnic et al., 1976). If repetitive flashes are used and, thus, the P680⁺ reduction by Z slowed down into the (sub) µsec range (e.g., Sonneveld et al., 1979), the rise in Chl a fluorescence yield in the µsec range is indicative, among other things, of the rate of electron flow from Z to P680⁺ because P680⁺ is known to be a quencher of fluorescence (Okayama and Butler, 1972; Butler et al., 1973). Therefore, P680⁺ reduction results in an increase in fluorescence yield. These rise kinetics are not influenced by HCO₃⁻* (Jurisnic et al., 1976), indicating that the P680⁺ reduction by Z is insensitive to HCO₃⁻*. The EPR signal Πᵥᵢ kinetics, monitoring the reduction of Z⁺ by the oxygen-evolving system (Blankenship et al., 1975), which were measured in an experiment using repetitive flashes, are also unchanged by CO₂ depletion (Jurisnic et al., 1976). However, the amplitude of the signal is decreased by 40% in the absence of HCO₃⁻*. One explanation for this decrease is that the dark time between the flashes (1 sec) was not long enough to reoxidize Q⁻ completely (the Q⁻ reoxidation in CO₂-depleted chloroplasts has a very slow component as mentioned previously). The P680* (i.e., P680 in the excited state) cannot transfer an electron to Q⁻, and there is no stable charge separation. For this reason, a non-negligible portion of Z is not oxidized after a flash, and the EPR Πᵥᵢ signal is expected to be lower in CO₂-depleted chloroplasts.

In our opinion, the absence of a significant change in the EPR Πᵥᵢ decay kinetics in CO₂-depleted chloroplasts, monitoring the reduction of Z⁺ by the oxygen-evolving system, rules out under these experimental conditions any significant role of HCO₃⁻* on the oxygen-evolving system, whereas the unchanged µsec fluorescence kinetics indicate that the electron donation to P680⁺ is not influenced by HCO₃⁻*.
F. Relationship between Herbicides and HCO$_3^-$

Experiments in which H$^{14}$CO$_3^-$ is added to CO$_2$-depleted chloroplasts and the H$^{14}$CO$_3^-$ binding monitored as a function of the H$^{14}$CO$_3^-$ concentration suggest that there is only one specific HCO$_3^-$ binding site per PSII reaction center (Stemler, 1977). Furthermore, it has been shown that the DCMU and HCO$_3^-$ binding sites are close together because H$^{14}$CO$_3^-$ removal by SiMo washing is greatly decreased by prior addition of DCMU (Stemler, 1977). A close spatial relationship between the HCO$_3^-$ and herbicide binding sites has now been described (Khanna et al., 1981; van Rensen and Vermaas, 1981b; Vermaas et al., 1982). Since herbicides are known to bind to a surface-exposed protein, very close to Q and B, and to be accessible to degradation by trypsin (Renger, 1976; Trebst, 1979; Arntzen et al., 1981), it is reasonable to assume that HCO$_3^-$ binds to the same or to an adjacent protein molecule. This explains why a HCO$_3^-$ interaction with the oxygen-evolving system or even with the donor side of PSII, although possible, is unlikely.

III. Postulated Effects of HCO$_3^-$ on the Donor Side of Photosystem II

There are some HCO$_3^-$ effects that are easily explained by assuming HCO$_3^-$ action on the water splitting system directly (see Stemler, Chapter 15, this volume). (For a recent review on water oxidation, S-states, and O$_2$ evolution, see Wydrzynski, Chapter 10, Vol. I, 1982.) However, here we argue that these data do not necessarily indicate a HCO$_3^-$ influence even on the donor side of PSII.

A. Effects on the S-States

When oxygen evolution in CO$_2$-depleted chloroplasts is measured as a function of flash number, the damping of the oscillation with a period of 4 (Kok et al., 1970) is faster without HCO$_3^-$ addition than with it (Stemler et al., 1974). This indicates that the miss parameter $\alpha$, the probability of not undergoing a net change in the charge accumulator M after a flash, is higher in the absence of HCO$_3^-$*. This might be due to a HCO$_3^-$* effect on the donor side, but is equally well explained by an effect on the acceptor side. If Q$^-$ is not fully reoxidized in the dark time between the flashes (1 sec), which is probably the case (Jursinic and Stemler, 1981), then a certain number of PSII centers (those of the type
P680$\cdot$Q$^-$) cannot transfer an electron from P680$^\ast$ to the first quinone acceptor as it is in Q$^-$ form and, therefore, no change is observed in those centers. Assuming that the slowly oxidizing Q$^-$ does not belong to a special type of PSII center, it is obvious that the high $\alpha$ value in CO$_2$-depleted chloroplasts can readily be explained by a HCO$_3^-\ast$ effect on the Q$^-$ reoxidation.

Furthermore, it is known that the time necessary to allow another turnover of the charge accumulator M (often denoted as the $S'_n \rightarrow S_{n+1}$ reaction rate) is extended by a factor of 10 or more by CO$_2$ depletion (600 $\mu$sec $\rightarrow$ 10 msec) (Stemler et al., 1974). This time necessary for "relaxation" (i.e., the $S'_n \rightarrow S_{n+1}$ reaction) is the time that it takes to open the PSII trap, i.e., to oxidize Q$^-$ or to reduce P680$^\ast$, whichever is slower. Since the Q$^-$ oxidation is slowed down by CO$_2$ depletion to a value of at least 4–10 msec (Jurisic et al., 1976; Siggel et al., 1977), it is probable that the slowing down of the $S'_n \rightarrow S_{n+1}$ reaction rate is caused by an effect of HCO$_3^-\ast$ on the reoxidation of Q$^-$ (Jurisic et al., 1976; Siggel et al., 1977). Under these circumstances, the very slow component of Q$^-$ reoxidation does not seem to show up in the rate of the $S'_n \rightarrow S_{n+1}$ reaction.

The kinetics of oxygen evolution ($S'_4 \rightarrow S_0$) as measured by an unmodulated Joliot electrode are not affected by HCO$_3^-\ast$ at pH 6.8 (Stemler et al., 1974). However, at pH 5.3, small differences in the kinetics of O$_2$ evolution were detected as stated in Chapter 15 ($t_{1/2} = 4.93 \pm 0.18$ msec, $+\text{HCO}_3^-\ast$; $t_{1/2} = 5.52 \pm 0.27$ msec, $-\text{HCO}_3^-\ast$) (Stemler, 1981); these differences are very small as compared to other HCO$_3^-\ast$ effects, and they could not be confirmed by the authors (Fig. 4).

Sodium formate (NaHCO$_2$), which seems to compete with HCO$_3^-\ast$ (N. Good, unpublished; Khanna et al., 1977; Vermaas and van Rensen, 1981), was shown to lengthen the relaxation time of $S'_6 \rightarrow S_3$ and $S'_6 \rightarrow S_3$ at pH 8.2, without affecting $S'_0 \rightarrow S_1$, $S'_1 \rightarrow S_2$, $S'_1 \rightarrow S_2$, and the "double hit" parameter $\beta$ (Stemler, 1980a). Stemler proposed that those S-state transitions that show an extended relaxation time in the presence of formate must result in momentary release and re-binding of CO$_2$ (see Stemler, Chapter 15, this volume). If formate under these conditions would be able to replace HCO$_3^-\ast$, then a higher $\alpha$ value should be obtained in the presence of formate than in the absence of formate, whereas $S'_0 \rightarrow S_1$ and $S'_1 \rightarrow S_2$ also should be slowed down because of the slowing down of Q$^-$ decay in the absence of HCO$_3^-\ast$. This was not observed. Therefore, we suggest that under these conditions (pH 8.2) formate may not be able to remove HCO$_3^-\ast$ from its binding site. The differences that were observed in the $S'_2 \rightarrow S_3$ and $S'_3 \rightarrow S_0$ reactions in the presence and absence of formate could also be explained by a formate effect, other than HCO$_3^-\ast$ removal, which affects $S_2$ and $S_3$ specifically. Bouges-
FIG. 4. Oxygen evolution, after the third flash, as a function of time in the presence (—) and the absence (— - -) of 10 mM NaHCO$_3$. Measurements were made with an unmodulated Joliot electrode. CO$_2$-free medium consisted of 50 mM Na phosphate, 100 mM NaHCO$_3$, 100 mM NaCl, 5 mM MgCl$_2$, pH 5.3, and CO$_2$-sufficient medium was 50 mM Na phosphate, 100 mM NaHCO$_3$, 10 mM NaHCO$_3$, 90 mM NaCl, 5 mM MgCl$_2$, pH 5.3. Xenon flashes, $\leq$ 10 µsec; number of experiments, 5. Note the absence of decrease in $t_{1/2}$ of the signal rise upon addition of CO$_2$. (Data of W. F. J. Vermaas and Govindjee, unpublished observations, 1980–1981.)

Bocquet (1980) suggested that two different electron donors, Z$_1$ and Z$_2$, donate electrons to P680 in parallel. Both of them are related to transitions in two S-states. Z$_1$ is connected to S$_0$ and S$_1$, and Z$_2$ is connected to S$_2$ and S$_3$. Thus, formate may slow down the Z$_2$ reduction but not the Z$_1$ reduction.

It is difficult to understand why at pH 8.2 formate does not affect S$_0' \rightarrow$ S$_1$ and S$_1' \rightarrow$ S$_2$ transitions, whereas at low pH those transitions are affected, but differentially (see Chapter 15). In Stemler's hypothetical scheme of CO$_2$ involvement in oxygen evolution, S$_0' \rightarrow$ S$_1$ and S$_1' \rightarrow$ S$_2$ would not be influenced by HCO$_3^-$ (Stemler, 1980a), and, therefore, this scheme does not explain his S$_0' \rightarrow$ S$_1$ and S$_1' \rightarrow$ S$_2$ data at pH 5.3 either. A possible explanation is that the Q$^-$ reoxidation is S-state dependent. It is known that the rate of Q$^-$ reoxidation by a back reaction is dependent on the S-state (Joliot et al., 1971; Lavergne and Etienne, 1982), but more experiments are needed to check if such a statement can explain the observed difference under these low pH conditions.
B. $H^{14}CO_3^-$* Binding Studies

Stemler (1980b) showed that the rate of $H^{14}CO_3^-$* binding to $CO_2$-depleted chloroplasts is pH independent in the first 2 mins. However, when the thylakoids are equilibrated for 5 min followed by $H^{14}CO_3^-$* addition, then the binding is faster at low pH than at high pH (pH 6.0 versus 7.8). These results were interpreted by Stemler (1980b) to suggest that the internal pH (which was assumed to be equal to the external pH after 5 min equilibration but not in the first 2 mins) rather than the external pH governs $HCO_3^-$* binding. Since the oxygen-evolving site is located on the inner side of the thylakoid membrane, an interaction of $HCO_3^-$* with the oxygen-evolving system was suggested (see Stemler, Chapter 15, this volume). However, $CO_2$-depleted chloroplasts are uncoupled by the depletion procedure (Khanna et al., 1977) and, thus, a fast pH equilibration (in the second range) between the inside and the outside of the thylakoid vesicle may be obtained. Furthermore, even in nondepleted (control) chloroplasts, the pH equilibration over the thylakoid membrane is rather rapid (rate constant of proton leakage: 0.73 sec$^{-1}$) (Khanna et al., 1980). This means that if the internal pH is important, differences in $H^{14}CO_3^-$* binding are expected to be lost within a couple of seconds. This was not observed. Further theoretical and experimental analyses are required, and, in our opinion, these data cannot yet be used to support $HCO_3^-$* binding to the inside of the thylakoid membrane.

IV. Conclusions

Although it has been assumed that $HCO_3^-$* may act on the donor side of PSII, perhaps even as a direct source of $O_2$ produced in the oxygen-evolving system (Warburg and Krippahl, 1958, 1960; Stemler, 1980a), there is an overwhelming amount of experimental observations now that support a major $HCO_3^-$* function between $Q$ and $PQ$ on the acceptor side of PSII (for earlier reviews, see Govindjee and van Rensen, 1978; Jordan and Govindjee, 1980; Vermaas and Govindjee, 1981a).

We believe that there are, as yet, no clear experimental results that point to any $HCO_3^-$* function on the donor side of PSII (Section III). In our opinion, the hypothetical scheme for a $HCO_3^-$* function in oxygen evolution as presented by Stemler (Chapter 15, this volume) is not based on convincing experimental data. We prefer a scheme in which $HCO_3^-$* binds to a site on or close to the herbicide binding protein, causing a conformational change in that protein that results in an allowance of
efficient electron transfer between Q and PQ (e.g., see Khanna et al., 1981; Vermaas and van Rensen, 1981). Clearly, more experiments are needed to understand the molecular mode of action and the function of the bicarbonate effect in intact systems.

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