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Minireview

Role of bicarbonate at the acceptor side of Photosystem II

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Abstract

Besides being the substrate for the carboxylation reaction of photosynthesis, CO_2 (bicarbonate) is required for the activity of Photosystem II (water plastoquinone oxido-reductase). It plays a role on the electron donor side as well as the electron acceptor side. In this contribution, attention will mostly be focused on the history of research into the effects of bicarbonate on electron flow reactions on the acceptor side. Donor side reactions are discussed in this issue by Alan Stemler.

Abbreviations: Chl – chlorophyll; DCMU (diuron) – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS II – Photosystem II; PQ – plastoquinone; Q_A – primary quinone electron acceptor of PS II; Q_B – secondary quinone electron acceptor of PS II

Introduction

In one of the earliest reviews about the effects of bicarbonate in Photosystem II (Govindjee and Jack van Rensen 1978), it was recalled that Jean Senebier (1782) was the earliest to note that the production of 'dephlogisticated air' (that is, oxygen) by plants requires the presence of 'fixed air' (carbon dioxide). Today, the role of carbon dioxide in photosynthesis is well understood; carbon dioxide is the substrate for the enzymatic reaction involved in this gas's reduction to carbohydrate. For a long time CO_2 was not thought to play a role in the light-based reactions of the photosynthetic process. A convenient example of such reactions is the Hill reaction (Robin Hill 1937; see David Walker, this issue), which allows a study to be made of oxygen evolution resulting from isolated broken chloroplasts illuminated in the presence of an artificial electron acceptor, such as ferricyanide or methyl viologen. The late Otto Warburg and Günter Krippahl (1958) discovered that this Hill reaction requires the presence of CO_2; in grana from kohlrabi leaves and using quinone or ferricyanide as an electron acceptor oxygen was evolved at a higher rate when the argon gas in the vessel contained 1.4% CO_2 (Figure 1; photographs of Otto Warburg appear elsewhere in this special issue in papers by Andrew A. Benson, Peter Homann, and Alan Stemler). This phenomenon was confirmed in the 1960s by many workers, e.g., the late Norman Good, the late Seikichi Izawa and the late Birgit Vennesland (for references, see the review by Danny Blubaugh and Govindjee 1988). However, there was little agreement as to the conditions necessary for the dependence of the Hill reaction on CO_2 and on the significance of such dependence. Norman Good (1963) studied the conditions under which a dependence of the Hill reaction on the presence of bicarbonate could be observed. Because he found that dependence on bicarbonate was correlated with the presence of other small anions during the depletion period, Good concluded that bicarbonate is the important species, not CO_2. This conclusion was later confirmed by Blubaugh and Govindjee (1986) by taking advantage of the pH dependence of the equilibrium

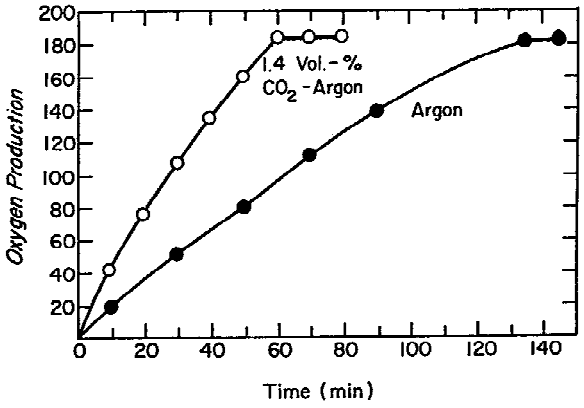

Figure 1. Experiments with grana suspended in 0.1% KCl (3 ml vessel). Electron acceptor, 2.1 mg quinone; gas phase, Argon or Argon + 1.4% CO_2 by volume. (after Warburg and Krippahl 1958). This experiment shows that CO_2 enhances the rate of the Hill reaction.

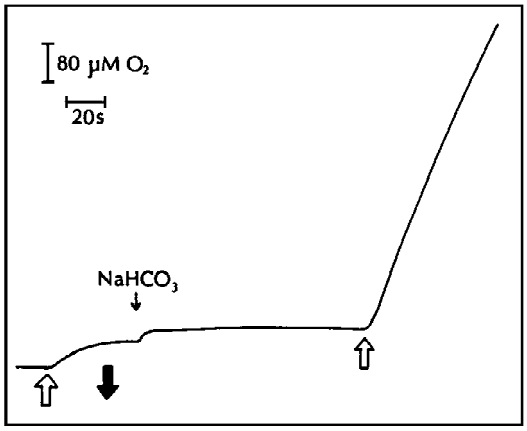

Figure 2. A typical example of the bicarbonate effect on a Hill reaction. Isolated chloroplasts (thylakoid membranes) were depleted of CO_2 in a medium at pH 5.8 in the presence of 25 mM formate while nitrogen gas was flushed over the suspension during 30 min. The recording of oxygen evolution was measured at pH 6.5 and is illustrated before and after the addition of 10 mM $NaHCO_3$. Arrow pointing upwards, light on; arrow downwards, light off.

ratio of $[CO_2]$ to $[HCO_3^-]$ to effectively hold one concentration constant while varying the other. The restoration of the Hill reaction was shown to be dependent only on the bicarbonate concentration over the pH range studied. CO_2 is the diffusing species and bicarbonate the active species.

Alan Stemler and Govindjee (1973) developed a reliable method to show the bicarbonate effect: they depleted isolated chloroplasts in a medium containing a high anion concentration at a pH below 6.0 and flushed nitrogen gas over the suspension in the dark. Because the pK of $H_2O + CO_2 \leftrightarrow H_2CO_3$ is 6.37, at low pH the equilibrium favors CO_2, which disappears by flushing with nitrogen gas. After depletion, the chloroplasts were transferred to a pH of 6.5 and the Hill reaction was measured after addition of an electron acceptor. The Hill reaction rate was low, but after addition of bicarbonate the rate was increased by several manifold. A typical example of such an experiment is illustrated in Figure 2 (J.J.S. van Rensen, unpublished).

Otto Warburg (1964) had considered the effect of bicarbonate on the Hill reaction as proof of his theory that oxygen arises from the splitting of CO_2 and not H_2O. One of the first to question the notion of direct splitting of water was Helmut Metzner (Metzner and Fischer 1969). Stemler (1980, 1982) has also always advocated a role of CO_2 in the process of water splitting in addition to the acceptor side effects. New results from the group led by Vyacheslav Klimov (see review by Klimov and Baranov 2001) may show that Stemler's views are correct in the end (Stemler, this issue).

Nevertheless, in 1975 Thomas Wydrzynski and Govindjee found that bicarbonate had an effect on the electron acceptor side of PS II. With this discovery, a new era of work began. The remaining history presented in this paper will concentrate on the research into this phenomenon. Figure 3 shows Govindjee with several of his former doctoral and postdoctoral associates, including the author, Stemler and Wydrzynski, (Photo, October, 1999).

Early evidence for the effect of bicarbonate at the acceptor side of Photosystem II

The first indication for an effect of bicarbonate on the reducing side of Photosystem II was observed by Wydrzynski and Govindjee (1975), who measured Chl *a* fluorescence induction kinetics in chloroplasts after CO_2 depletion. The variable Chl *a* fluorescence monitors the redox state of Q_A; Q_A is a quencher of fluorescence, whereas Q_A^- is not (Duysens and Sweers 1963). Therefore, a rapid accumulation of Q_A^- due to an inhibition of electron transport beyond Q_A is easily detected by fluorescence induction measurements. CO_2 depletion causes a fast increase in the variable fluorescence yield, similar but not identical to that observed in normal chloroplasts in the presence of the herbicide DCMU (Wydrzynski and Govindjee 1975). DCMU is known to block the reoxidation of Q_A^- by the secondary quinone acceptor Q_B (Figure 4).

Figure 3. Two photographs of Govindjee's research group gathered at his retirement in October, 1999, at Urbana, Illinois, USA. From left to right in (a): Jack van Rensen, Thomas Wydrzynski, Julian Eaton-Rye, Alan Stemler, Govindjee, and Rita Khanna. Photograph by Jin Xiong. (b) Barbara Zilinskas (who had worked on the partial reactions of photosynthesis, and antibodies against PS II in the 1970s), Alan Stemler, Rita Khanna, Govindjee, Jin Xiong, Julian Eaton-Rye, Paul Jursinic, and Thomas Wydrzynski. The little girl (four years old) in both the pictures is Govindjee's granddaughter Sunita Christiansen. Photograph taken by Rajni Govindjee. Others who had worked with Govindjee, in his lab, at Urbana, on the bicarbonate effect in PS II, but are missing from the photographs, are Danny Blubaugh, Jinchiang Cao, G. Sarojini, Wim Vermaas, and Chunhe Xu.

During a sabbatical in 1976 with Louis Duysens' research group in Leiden, Govindjee studied the DCMU-induced chlorophyll *a* fluorescence increase as a function of the number of pre-illuminating flashes and found that the binary oscillations, normally observed in Q_A^- reoxidation, were absent in CO_2-depleted chloroplasts (Govindjee et al. 1976). The measurement of chlorophyll *a* fluorescence yield after pre-illuminating flashes became an important tool to study the bicarbonate effect, especially in Govindjee's group. The decay of the chlorophyll *a* fluorescence yield after a saturating flash monitors the reoxidation of Q_A^-. Detailed information about the effect of CO_2 depletion was obtained by measuring the decay of the chlorophyll *a* fluorescence yield after various numbers

of short saturating flashes. With this technique, the following events are monitored.

After an odd number of flashes:

$$Q_AQ_B \overset{h\nu}{\rightarrow} Q_A{}^-Q_B \rightarrow Q_AQ_B{}^-$$

After an even number of flashes:

$$Q_AQ_B{}^- \overset{h\nu}{\rightarrow} Q_A{}^-Q_B{}^- \rightarrow Q_AQ_B{}^{2-};$$

$Q_B{}^{2-}$ becomes protonated and exchanges with the PQ pool: $Q_AQ_B{}^{2-} + 2H^+ + PQ \rightarrow Q_AQ_B + PQH_2$

Govindjee et al. (1976) found no differences in the fluorescence yield measured 160 ms after the last of a various number of flashes in control and in CO_2-depleted chloroplasts to which bicarbonate was added. In CO_2-depleted chloroplasts, however, they found little effect on the fluorescence intensity measured 160 ms after one or two flashes, but a much higher fluorescence after three or more flashes. It was reported by Paul Jursinic and Alan Stemler (1982) that the decay of fluorescence after one or many flashes was slower in depleted samples in both the 50 μs to 10 ms range and the 10 ms to 5 s time range. In CO_2-depleted samples, the half-time of the decay after one flash was in the short-term about 6 ms, and in the long-term about 1–2 s (Jursinic is shown in Figure 3b) Howard Robinson et al. (1984) measured fluorescence decay in a time range up to 10 ms and showed that the half-time of the $Q_A{}^-$-decay after one flash was 1.2 ms in CO_2-depleted chloroplasts and 230 μs in control chloroplasts: a five-fold difference. After three or more flashes, half-times were 13 ms for bicarbonate-depleted and 360 μs for the control: a 36-fold slower decay in bicarbonate-depleted samples. Although the absolute values of the rates of $Q_A{}^-$-decay in this type of experiment depend on the conditions of the experiment, it was clear that there is a smaller inhibition of CO_2 depletion on the $Q_A{}^-$ reoxidation by Q_B or by $Q_B{}^-$ but a much larger inhibition of the protonation of $Q_B{}^{2-}$ and/or exchange of Q_BH_2 with the plastoquinone pool. In CO_2-depleted chloroplasts three electrons can be stored so that $Q_A{}^-Q_B{}^{2-}$ is produced.

Binding sites of bicarbonate

The site of inhibition by CO_2-depletion was also determined by studying its effect on various parts of the electron transport chain (Rita Khanna et al. 1977; Khanna can be seen in Figures 3a and b). There was no effect of CO_2 depletion on electron transport from reduced diaminodurene to methyl viologen, indicating the absence of an effect on Photosystem-I-dependent electron transport. A large bicarbonate effect was demonstrated on the electron flow from water to oxidized diaminodurene in the presence of dibromothymoquinone (DBMIB), indicating an effect before the plastoquinone pool. Since the electron flow from water to silicomolybdate in the presence of DCMU was not affected by CO_2 depletion, it was concluded that the bicarbonate effect was located between Q_A and the PQ pool. It was reported, however, that silicomolybdate-supported oxygen evolution in the presence of DCMU was inhibited by formate and bicarbonate (Fred Crane and Rita Barr 1977; Jursinic and Stemler 1986). From the absence of a bicarbonate effect on electron transport in trypsin-treated chloroplasts, in which ferricyanide accepts electrons directly at the Q_A site (Jack van Rensen and Wim Vermaas 1981), it was again concluded that the location of the bicarbonate effect was between Q_A and the PQ pool.

The localization of the bicarbonate effect between Q_A and the plastoquinone pool was further concluded from the interaction of bicarbonate (or formate) with Photosystem II inhibiting herbicides. Van Rensen and coworkers (van Rensen and Vermaas 1981; Khanna et al. 1981; Snel and van Rensen 1983) studied the interaction of bicarbonate and herbicides through their effects on electron transport in isolated chloroplasts. By adding various concentrations of bicarbonate to CO_2-depleted chloroplasts, various rates of restoration of the Hill reaction were obtained. It was demonstrated that the effect of bicarbonate on thylakoid membranes shows Michaelis–Menten kinetics and that the thylakoid system can be treated like an enzyme *versus* bicarbonate, the substrate. 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 mM formate is present in the reaction medium, the apparent K_d appears to be about 1 mM bicarbonate, the same value was reported by Stemler and Judith Murphy (1983). The K_d for bicarbonate depends on the presence of both formate and of herbicides: in the presence of low concentrations of formate the apparent K_d decreases, approaching 80 μM $NaHCO_3$ in the absence of formate, and in the presence of urea, triazine or phenol-type herbicides, the K_d for bicarbonate increases by at least twofold (Stemler and Murphy 1983; Snel and van

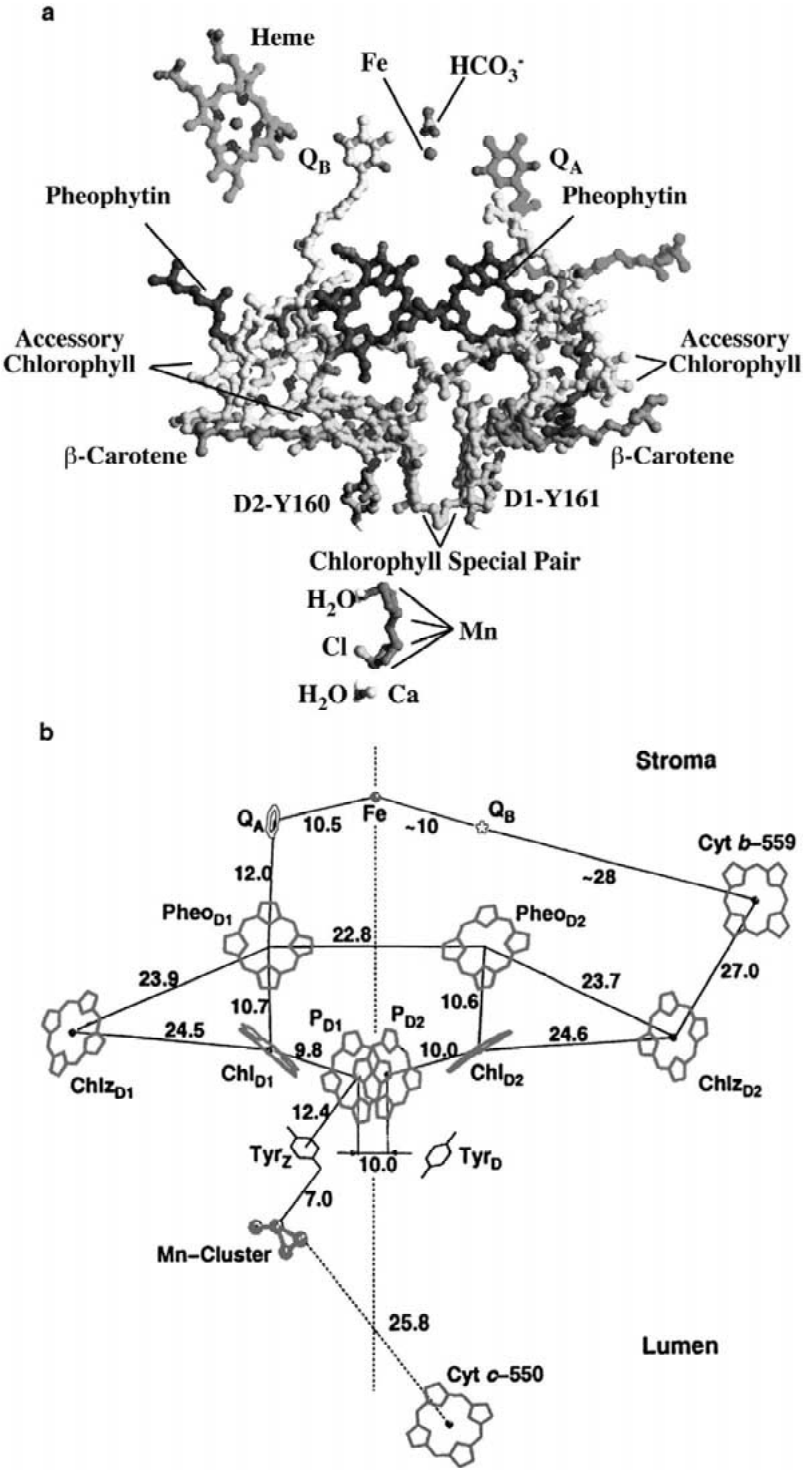

Figure 4. Top: the modeled cofactors in the PS II reaction center (from Xiong et al. 1998b; with kind permission from the authors). Bottom: chromophores and distances between them (from Zouni et al. 2001; with permission from H.T. Witt). Courtesy of Govindjee and of Jin Xiong. For a color version of this figure, see section in the front of the issue.

Rensen 1984). This means that these herbicides decrease the apparent affinity of the thylakoid membrane for bicarbonate. Also, it was observed that chloroplasts isolated from atrazine-resistant plants have a lower affinity for bicarbonate than wild-type ones (Khanna et al. 1981). These resistant plants have an alteration in the amino acid composition of the D1 protein: serine 264 is exchanged for glycine.

The above arguments led strongly to the suggestion that the binding sites of bicarbonate are located on the D1 protein of Photosystem II. Hartmut Michel and Johann Deisenhofer (1988) compared the primary structure of the L and M polypeptides of the bacterial reaction centers with the D1 and D2 polypeptides of Photosystem II and suggested that glutamate in bacteria is replaced by bicarbonate in PS II as a ligand to the nonheme iron. Interestingly, it was demonstrated by R. Shopes et al. (1989) that the bicarbonate effect is absent in anoxygenic photosynthetic bacteria. Wim Vermaas and William (Bill) Rutherford (1984) demonstrated that formate addition to thylakoids increases the amplitude of the $g = 1.82$ EPR signal of $Q_A^- Fe^{2+}$ 10-fold (see also Jonathan Nugent et al. 1988). A formate/bicarbonate effect was established clearly by measurements of EPR spectra of the Q_A^-Fe–Q_B complex with and without bicarbonate (Simon Bowden et al. 1991). The Mössbauer spectrum of Fe was affected significantly by formate and was returned to its original on re-addition of bicarbonate, indicating that Fe is a key element in the binding of formate that is displaced by bicarbonate (Bruce Diner and Vasili Petrouleas 1987; Semin et al. 1990). A Fourier transform infrared (FTIR) difference spectroscopy study using ^{13}C-labeled bicarbonate established that bicarbonate is a bidentate ligand of the nonheme iron (Hienerwadel and Catherine Berthomieu 1995). Examining the effects of a number of carboxylate anions on the EPR signals associated with the non-heme iron, Petrouleas et al. (1994) observed that glycolate, glyoxylate, and oxalate compete with NO, formate and bicarbonate for binding to the nonheme iron. Further work, mainly by Govindjee's group, led to the conclusion that there are two binding sites (Blubaugh and Govindjee 1988) for bicarbonate in the D1 protein of PS II: one on the nonheme iron and the other near arginine 257 (Xiong et al. 1996, 1998a; van Rensen et al. 1999; Xiong is visible in Figure 3b). One of these locations is illustrated in Figure 4 (top).

Function of bicarbonate on the acceptor side of Photosystem II

The functions of bicarbonate in PS II are not yet clear. There have been early suggestions that bicarbonate and formate may interfere with protonation reactions near Q_B (see a review by Govindjee and van Rensen 1993). It is possible that H_2CO_3 is involved in the protonation of Q_B^{2-} or its proteinaceous environment, since the pK_a of (CO_2 + H_2O) is 6.37 at 25 °C. $CO_2/HCO_3^-/CO_3^{2-}$ could serve as a proton shuttle between Q_B and the external aqueous phase. Formate is not able to function in such a way, because the pK_a of formate is 3.8. Evidence for such a function was obtained by van Rensen et al. (1988), Julian Eaton-Rye and Govindjee (1988) and Chunhe Xu et al. (1991). (Eaton-Rye is to be seen in Figures 3a and b.)

Because bicarbonate is liganded to the nonheme iron, an additional role of bicarbonate may be to stabilize the Q_A-Fe-Q_B structure. Upon the removal of bicarbonate, the distance between Q_A and Q_B may be altered, slowing down the rate of electron flow between these two quinone acceptors (van Rensen et al. 1999). Such an influence of bicarbonate does not explain, however, why the decay of the fluorescence yield after three or more flashes is much more strongly affected than after the first flash.

Current knowledge of the bicarbonate effect is almost exclusively based on experiments carried out with isolated thylakoid membranes. The observation of a bicarbonate effect on Photosystem II *in vivo* is difficult to distinguish from the obvious requirement for CO_2 in the Calvin–Benson cycle. There is only a small number of reports about an effect of bicarbonate on PS II *in vivo*. A few observations on *in vivo* effects on Photosystem II are given by Gyözö Garab et al. (1983), Dierk Mende and Wolfgang Wiessner (1985), and Fatma El-Shintinaway and Govindjee (1990).

Under conditions in which photosynthesis can proceed well, enough bicarbonate is probably bound to PS II in order for PS II to function normally. However, under stress conditions (e.g., drought, high light intensity, high temperature) the stomata may close, which would lead to a decrease in internal CO_2 concentration that may limit the activity of PS II. It has been suggested several times that bicarbonate may be involved in the process of photoinhibition (for references, see van Rensen et al. 1999).

Concluding remarks

At the evolutionary time that a PS-II-type reaction center incorporated a water-oxidizing machinery using water as a source for electrons, the atmosphere contained a high concentration of CO_2. It is conceivable that with abundant amounts of carbon dioxide, CO_2 was used in some way to make PS II functional. It will be challenging to search for those functions in the future. The resolution of the structure of the PS II reaction center (see Zouni et al. 2001) will be a great help in the design of new experiments, both at the acceptor as well as at the donor side of PS II. We anxiously await the final verdict on the recent suggestions of V. Klimov, Charles Dismukes, and others (see e.g. Dismukes et al. 2001) that bicarbonate may indeed be involved on the donor side of PS II. This is a new beginning to indeed test the idea of the role of bicarbonate on the donor side of PS II (also see A.J. Stemler, this issue), in addition to the established role of bicarbonate on the acceptor side of PS II discussed here.

Acknowledgments

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