

Bicarbonate Does Not Influence Electron Transfer to the Reaction Center Chlorophyll *a* of Photosystem II

Measurements by Chlorophyll *a* Fluorescence Rise in Microseconds

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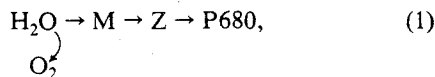
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Bicarbonate ions (or CO₂) dramatically influence the electron transport in the water-plastoquinone oxido-reductase, the so-called photosystem II (PS II) [1–3]. Removal of CO₂ or bicarbonate, by the formate (or the nitrite) addition, reversibly slows down the electron transfer from the reduced Q_A, where Q_A is the first plastoquinone electron acceptor of PS II, to the plastoquinol pool [4–6]. Although CO₂ may be the diffusing species, HCO₃⁻ is the active species that stimulates the electron flow [7]. The requirements of HCO₃⁻ for the electron flow in the Hill reaction has been suggested by experiments that show that the residual activity in the bicarbonate-depleted membranes is due to the residual amount of bicarbonate bound to the membrane [8].

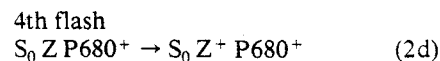
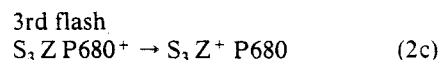
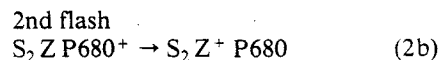
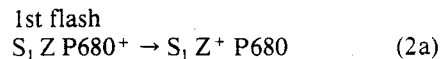
In contrast to the idea that bicarbonate plays a role on the electron acceptor side of photosystem II [1–3], but consistent with the Warburg's ideas [9], Stemler [10] and Metzner et al. [11] suggested that bicarbonate may be involved in the oxygen evolution process. Metzner et al. [11] claimed to have observed a slight enrichment of evolved O₂ in ¹⁸O as compared to that in water when C¹⁸O₂ was present, and argued for a role of CO₂ in O₂ evolution. However, more recent experiments have failed to confirm this result [12]. Furthermore, bicarbonate depletion had no effect on the kinetics of O₂ evolution after a flash [13]. Jursinic et al. [5] examined directly the effects of bicarbonate depletion on the electron donor (the oxygen-evolving) side of photosystem II:



where P680 is the reaction center chlorophyll *a*, Z is a tyrosine residue of the protein, and M is the Mn-containing oxygen-evolving complex (see review [14]). In a repetitive flash measurement, the half-time of the decay of ESR signal II very fast, monitoring the electron flow from M to Z, was unaffected (*t*_{1/2} = 800–900 μs) [5]. Similarly, chlorophyll *a* fluorescence rise kinetics, using the protocol of Den Haan et al. [15], showed no effect of bicarbonate depletion [5]. This suggested to the authors that the Z to P680⁺ reaction was independent of bicarbonate. It had been suggested that chlorophyll *a* fluorescence rise monitors Z P680⁺ → Z⁺ P680 reaction since P680⁺ is a quencher [16].

Unfortunately, bright light flashes also create carotenoid triplets, and the decay of such triplets also increases fluorescence in the microsecond range used in the above studies [15]. Sonneveld et al. [17] have shown that the time of electron flow from Z to P680, as monitored by chlorophyll *a* fluorescence rise, and corrected for the triplet decay, oscillates with flash number, as it depends upon the redox states (S) of M. In dark-adapted samples, M exists in S₁, and Z to P680 reaction is very fast (≈ 20 ns) after the first flash; slower reaction (≈ 200 ns) is observed after the second and the third flashes when M is in the S₂ or the S₃ states before the flash. The reaction is fast again after the fifth flash as M again starts in S₁. These conclusions have been confirmed

directly by monitoring the absorbance changes of P680 [18]. In this paper, we present data on the effect of bicarbonate depletion on the Z P680⁺ → Z⁺ P680 reaction, as measured by chlorophyll *a* fluorescence rise, corrected for carotenoid triplet decay, in the submicrosecond to microsecond range following single-turnover flashes. No effect of bicarbonate depletion was observed on this reaction after flashes 1, 2, 3, and 4, proving the absence of bicarbonate effect on the following reactions:



Here, S_{*n*} stands for the redox state of M, P680⁺ is created by light, and the electron acceptor side has been ignored for brevity and simplicity.

Thylakoid membranes were prepared from pea leaves as described elsewhere [7]. The chlorophyll concentration was determined by the spectrophotometric method in [19]. Thylakoid membranes were suspended in a CO₂(HCO₃⁻)-free treatment medium (50 mM Na phosphate, 100 mM NaCl, 5 mM MgCl₂ and 100 mM NaHCO₂, pH 5.5) and depleted of bicarbonate as described in [8]. These treated samples were resuspended in a reaction medium that contained the same medium except that the pH was 6.5. In the control samples, CO₂-sufficient medium was used, and the sodium formate was omitted from both the treatment and the reaction medium. The reversibility of the bicarbonate-depletion effect was tested by adding 5 mM NaHCO₃ to the formate containing CO₂-depleted samples. The final concentration of chlorophyll was 4 μg ml⁻¹ of suspension.

Electron transport from Z to P680⁺ was measured by monitoring chlorophyll *a* (Chl *a*) fluorescence rise in the microsecond range as described in detail in [20]. Fluorescence rise in the experimental samples was divided by fluorescence rise in samples that were preilluminated in the presence of 10 μM DCMU and 10 mM NH₂OH. Under

these conditions, photosystem II does not have any photochemically related Chl *a* fluorescence rise, and the observed transient kinetics in the μs range are due to the disappearance of the carotenoid triplet quencher only. Thus, this method corrects for changes due to the triplets. The rate of the appearance of the high fluorescence state P680 Q_A^- , under the experimental conditions used, depends upon the convolution of the rate of reduction of P^+680 by *Z* and the rate of photochemical activation, i.e., the formation of the P680 Q_A^- state. Since the light intensity chosen for these experiments was not saturating, the fluorescence rise does not directly measure the reduction rate of P680^+ by *Z* but is clearly dependent upon it. The observed larger fluorescence increase (ΔF) from 0 to 3.5 μs after the first flash and the differential kinetics compared with that after the second and subsequent flashes assure us that ΔF is an indicator of the *Z* to P680^+ reaction [20], and that difference of the reaction rate (30 ns vs. 200 ns) in the ns range can be monitored through fluorescence. No attempt was made here to deconvolute the true reaction kinetics.

In order to be sure that the samples we were using for our measurements on the electron flow from *Z* to P680^+ were indeed bicarbonate-depleted, we measured the decay of Chl *a* fluorescence (representing electron flow from Q_A^- to Q_B). Figure 1 (right panel, after flashes 1–4) shows that bicarbonate depletion indeed slowed the electron flow from Q_A^- to Q_B , as published elsewhere [5, 6]. Furthermore, this inhibition was reversible as the addition of 5 mM Na bicarbonate reversed the effect fully (Fig. 1, left panels, after flashes 1–4). Data for control (untreated) samples were superimposed on these data (not shown).

Figure 2 shows the time curve over 3.5 μs of the relative chlorophyll *a* fluorescence yield at 690 nm in bicarbonate depleted (right) and bicarbonate-restored (left) samples. The ΔF is highest after the first flash in both cases; this ΔF decreased somewhat in both cases for flash 2, followed by a small decrease after flash 3. Both the samples showed a flash pattern similar to that published earlier for normal thylakoid membranes, but quite different from that published for Cl^- -depleted

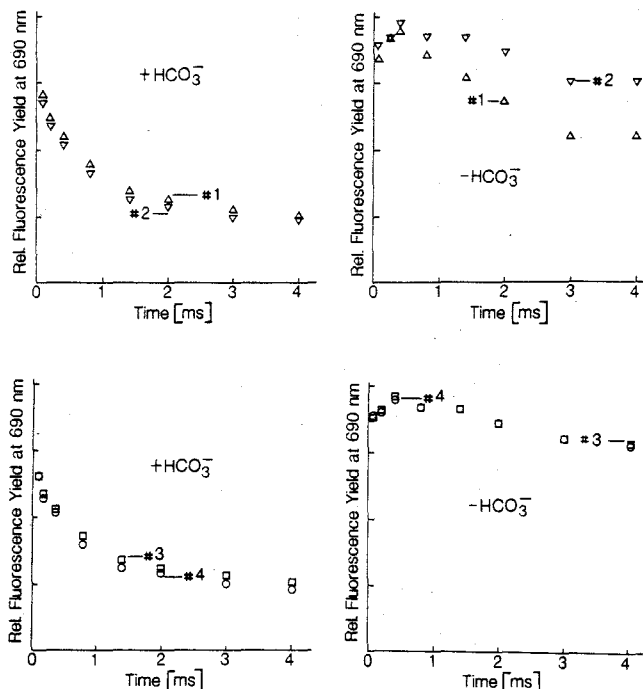


Fig. 1. Decay of variable fluorescence after actinic flashes spaced at 0.5 s; Δ after one flash, ∇ after two flashes, \square after three, \circ after four flashes; right: CO_2 -depleted, left: after addition of 5 mM NaHCO_3

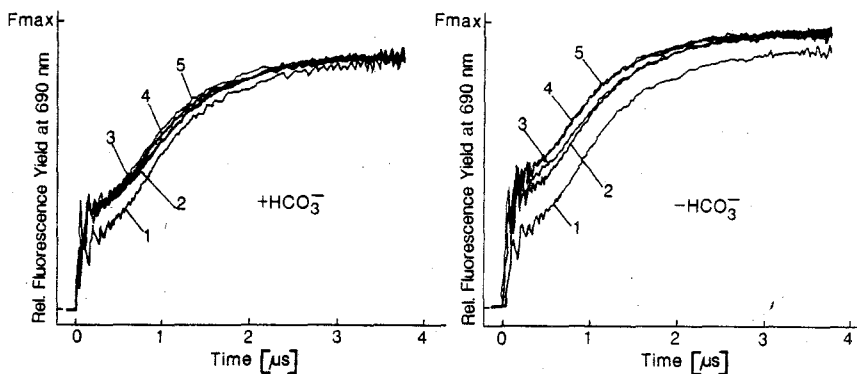


Fig. 2. Fluorescence rise in the μs time range during actinic flashes spaced at 0.5 s. Numbers along the curves are the flash numbers; right: CO_2 -depleted, left: after addition of 5 mM NaHCO_3

samples which show a dramatic lowering of ΔF after the 2nd and the 3rd flashes [20]*.

In order to more easily evaluate the bicarbonate effect on the *Z* to P680^+ reaction and on the production of P680 Q_A^- state as a function of the redox state of the oxygen-evolving complex, we show (Fig. 3) a comparison on each flash (1 through 4) of Chl *a* fluorescence rise in the μs time range, during a

xenon flash, with and without bicarbonate. Equations 2 (a)–2 (d) show the steps during flash 1 through 4, respectively. Figure 3 shows that bicarbonate did not have significant effect on any of the reactions by which P680^+ reduction occurred. Furthermore, we can also exclude a major inhibition in the later reactions of the oxygen-evolving complex, since any dramatic effect on the electron donor

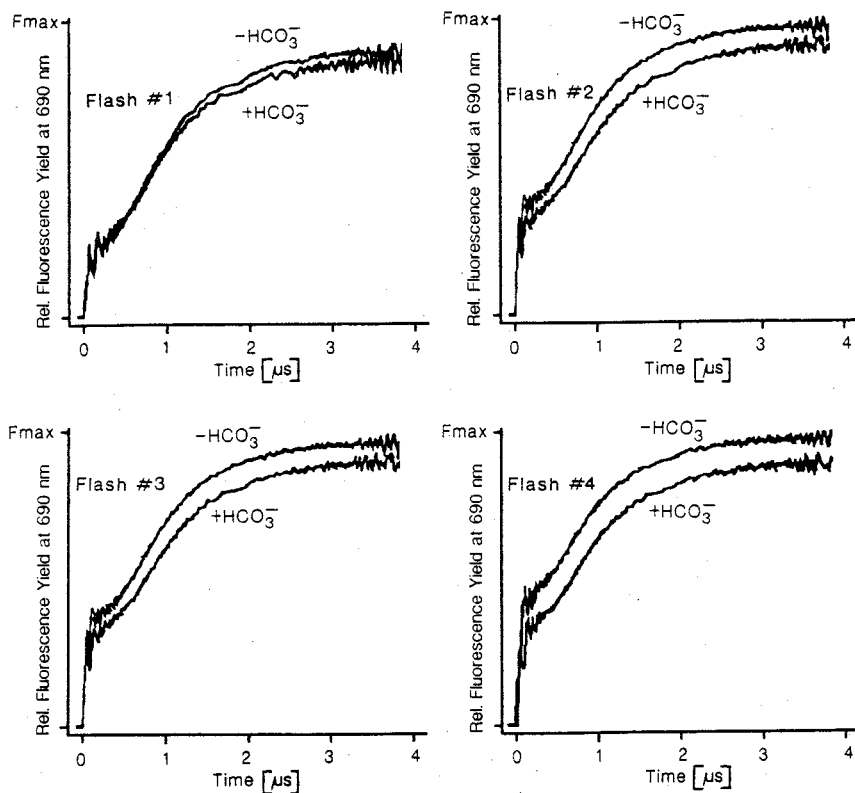


Fig. 3. Comparison of the fluorescence rise in the μs time range during xenon flashes number one, two, three, and four. The flashes were spaced at 0.5 s. $-\text{HCO}_3^-$: CO_2 -depleted; $+\text{HCO}_3^-$: after addition of 5 mM NaHCO_3

side would affect these steps, as observed in the case of Cl^- depletion [20]. Not only was no significant effect of bicarbonate observed on ΔF , but also the $t_{1/2}$ of the rise of the Chl a fluorescence, which represents a convolution of Z to P680^+ and the rate of formation of P680 Q_A^- , remained unaffected (approximately $0.9 \pm 0.1 \mu\text{s}$) in all cases. In summary, we have established here that neither the rate of electron flow from Z to P680^+ nor the rate of formation of P680 Q_A^- is significantly af-

ected by bicarbonate depletion for any of the redox states of the oxygen-evolving complex.

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1. Govindjee, van Rensen, J. J. S.: *Biochim. Biophys. Acta* 505, 183 (1978)
2. Govindjee, Eaton-Rye, J. J.: *Photosynth. Res.* 10, 365 (1986)
3. Blubaugh, D., Govindjee: *ibid.* 19, 85 (1988)

4. Govindjee, et al.: *Biochim. Biophys. Acta* 449, 602 (1976)
5. Jursinic, P., et al.: *ibid.* 440, 322 (1976)
6. Eaton-Rye, J., Govindjee: *ibid.* 935, 237 (1988)
7. Blubaugh, D., Govindjee: *ibid.* 848, 147 (1986)
8. Blubaugh, D., Govindjee: *ibid.* 936, 208 (1988)
9. Warburg, O.: *Ann. Rev. Biochem.* 33, 1 (1964)
10. Stemler, A., in: *Photosynthesis*, Vol. II, p. 513 (Govindjee, ed.). New York: Academic Press 1982
11. Metzner, H., et al.: *Biochim. Biophys. Acta* 548, 287 (1979)
12. Guy, R. D., et al., in: *Progress in Photosynthesis Research*, Vol. 3, p. 597 (J. Biggins, ed.). Dordrecht: Nijhoff 1987
13. Vermaas, W. F. J., Govindjee, in: *Photosynthesis*, Vol. II, p. 541 (Govindjee, ed.). New York: Academic Press 1982
14. Vermaas, W. F. J., in: *Techniques and New Developments in Photosynthesis Research* (J. Barber, ed.). New York: Plenum (in press)
15. Den Haan, G. A., et al.: *Biochim. Biophys. Acta* 368, 409 (1974)
16. Butler, W. L.: *Accnts. Chem. Res.* 6, 177 (1972)
17. Sonneveld, A., et al.: *Biochim. Biophys. Acta* 548, 536 (1979)
18. Brettel, K., et al.: *ibid.* 766, 403 (1984)
19. MacKinney, G.: *J. Biol. Chem.* 140, 315 (1941)
20. Itoh, S., et al.: *Biochim. Biophys. Acta* 766, 612 (1984)

*** Note added in Proof**

The larger difference observed between flash 1 and 2 for ΔF at $3.5 \mu\text{s}$ in the bicarbonate-depleted case versus that in the bicarbonate-restored case (Fig. 2) and the differences observed between ΔF s at $3.5 \mu\text{s}$ in the plus and minus bicarbonate cases after flashes 2, 3, and 4 (Fig. 3) are due to the slowing down of electron flow beyond Q_A^- upon bicarbonate depletion, noted in Fig. 1.