Bicarbonate Does Not Influence Electron Transfer to
the Reaction Center Chlorophyll \( \alpha \) of Photosystem II

Measurements by Chlorophyll \( \alpha \) Fluorescence Rise in Microseconds

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Bicarbonate ions (or \( \text{CO}_2 \)) dramatically influence the electron transport in the water-plastoquinone oxido-reductase, the so-called photosystem II (PS II) [1–3]. Removal of \( \text{CO}_2 \) or bicarbonate, by the formate (or the nitrite) addition, reversibly slows down the electron transfer from the reduced \( \text{Q}_A \), where \( \text{Q}_A \) is the first plastoquinone electron acceptor of PS II, to the plastoquinol pool [4–6]. Although \( \text{CO}_2 \) may be the diffusing species, \( \text{HCO}_3^- \) is the active species that stimulates the electron flow [7]. The requirements of \( \text{HCO}_3^- \) 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 \( \text{O}_2 \) (as \( ^{18}\text{O} \)) compared to that in water when \( ^{18}\text{O}_2 \) was present, and argued for a role of \( \text{CO}_2 \) in \( \text{O}_2 \) evolution. However, more recent experiments have failed to confirm this result [12]. Furthermore, bicarbonate depletion had no effect on the kinetics of \( \text{O}_2 \) 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:

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\begin{align*}
\text{H}_2\text{O} & \rightarrow \text{M} \rightarrow Z \rightarrow \text{P680}, \\
\text{O}_2 & \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \qua
these conditions, photosystem II does not have any photochemically related Chl \( a \) fluorescence rise, and the observed transient kinetics in the \( \mu s \) 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 \( Q_{\lambda}^- \), under the experimental conditions used, depends upon the convolution of the rate of reduction of P680 by Z and the rate of photochemical activation, i.e., the formation of the P680 \( Q_{\lambda}^- \) 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 (\( \Delta F \)) from 0 to 3.5 \( \mu s \) after the first flash and the differential kinetics compared with that after the second and subsequent flashes assures us that \( \Delta F \) 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 \( Q_{\lambda} \) to \( Q_{b} \)). Figure 1 (right panel, after flashes 1–4) shows that bicarbonate depletion indeed slowed the electron flow from \( Q_{\lambda} \) 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 \( \mu s \) of the relative chlorophyll \( a \) fluorescence yield at 690 nm in bicarbonate depleted (right) and bicarbonate-restored (left) samples. The \( \Delta F \) is highest after the first flash in both cases; this \( \Delta F \) 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 samples which show a dramatic lowering of \( \Delta F \) 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 \( Q_{\lambda}^- \) 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 \( \mu s \) 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

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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$_3$.

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*Note added in Proof*

The larger difference observed between flash 1 and 2 for ΔF at 3.5 μs in the bicarbonate-depleted case versus that in the bicarbonate-restored case (Fig. 2) and the differences observed between ΔFs at 3.5 μs 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$_\lambda$ upon bicarbonate depletion, noted in Fig. 1.