# THE EFFECT OF pH AND FLASH FREQUENCY ON ELECTRON TRANSFER THROUGH THE QUINONE ACCEPTOR COMPLEX OF PS II IN BICARBONATE DEPLETED OR ANION INHIBITED THYLAKOID MEMBRANES

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## 1. INTRODUCTION

Electron transfer through the plastoquinone acceptors of photosystem II (PS II) is inhibited by a number of monovalent anions. These include formate, nitrite and acetate [1-3]. However, the $HCO_3^-$ anion possesses the unique ability to reverse this inhibition. We have suggested that the ability of $HCO_3^-$ to facilitate electron flow may involve participation in the protonation reactions of the two-electron gate [4]. To test this hypothesis we have studied the kinetics of $Q_A^-$ reoxidation in control, anion inhibited/$HCO_3^-$-depleted and $HCO_3^-$-restored samples as a function of pH and flash frequency. Below, we present some preliminary findings from this work.

## 2. MATERIALS AND METHODS

Thylakoid membranes were prepared from spinach and anion inhibited/$HCO_3^-$-depleted samples (hereafter referred to as treated membranes) were obtained by a dark incubation for 60 min in a $CO_2$-free buffer. Detailed methods for these procedures have been published in ref. [5]. The treatment buffer contained 300 mM sorbitol, 25 mM sodium formate, 10 mM NaCl, 5 mM $MgCl_2$ and 10 mM sodium phosphate (pH 6.0). The chlorophyll (Chl) concentration was 250 µM. The reaction medium contained 100 mM sorbitol, 10 mM sodium formate, 10 mM NaCl, 5 mM $MgCl_2$, 20 mM buffer (MES, pH 6.0-6.5; HEPES, pH 6.7-8.0), 100 µM methyl viologen and 0.1 µM gramicidin. All measurements were made on a sample diluted to contain 5 µM Chl in a final volume of 100 ml in a dark stirred vat. A flow cuvette was filled from the vat under computer control.

Restored membranes were obtained by adding 5 mM $HCO_3^-$ to a 2 ml aliquot of the treated stock. After a 2 min dark incubation these membranes were transferred to the reaction medium which also contained 5 mM $HCO_3^-$. Control membranes were obtained by omitting formate from the treatment and reaction media and not $CO_2$-depleting these buffers. In the case of the control the incubation pH was also raised to pH 7.5. Following isolation, the thylakoid membranes were maintained at $20^0$ C in all cases.

The kinetics of decay of variable Chl _a_ fluorescence at 685 nm (indicating oxidation of $Q_A^-$ by either $Q_B^-$ or $Q_B$) were measured by a weak measuring flash after each of a series of actinic flashes. This technique and an identical instrument have previously been described [6]. $Q_A$ and $Q_B$ are, respectively, the primary and secondary quinone acceptors of PS II.

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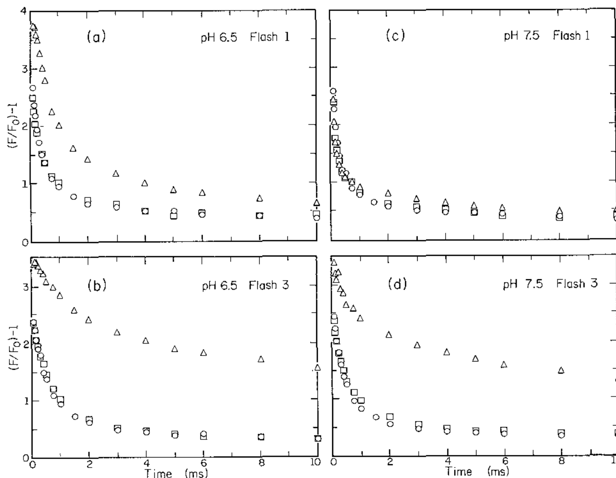


FIGURE 1. Decays of variable Chl a fluorescence after 1 or 3 actinic flashes, given at 1 Hz, at pH 6.5 and pH 7.5. Treated membranes are shown as △, restored membranes are shown as O and control membranes as □. $F_0$ is the Chl a fluorescence yield from the measuring flash with all $Q_A$ oxidized and F is the yield at the indicated time after the actinic flash. Half-times for $Q_A^-$ oxidation were determined as in [5]. The approximate half-times were for (a): treated membranes, 2.2 ms; restored membranes, 520 µs; control membranes, 550 µs; (b): treated, 20 ms; restored, 520 µs; control, 650 µs; (c): treated, 360 µs; restored, 340 µs; control, 320 µs and (d): treated, 15 ms; restored, 450 µs; control, 500 µs (after Eaton-Rye and Govindjee [8]).

## 3. RESULTS AND DISCUSSION

Chlorophyll a fluorescence decays, monitoring the oxidation of $Q_A^-$, following 1 or 3 single turnover actinic flashes are presented in Fig. 1. The data are shown at two pH values. At pH 7.5 the decay of $Q_A^-$ to $Q_A$, after a single flash, is shown to be almost independent of the $HCO_3^-$-depletion/anion inhibition treatment. However, at pH 6.5, treated membranes show a slower decay of $Q_A^-$ to $Q_A$. This result suggests that at pH 6.5, $Q_A^-$ is stabilized in the treated case. We have also observed that the back reaction of $Q_A^-$ with the $S_2$ state of the oxygen evolving complex, in the presence of 5 µM DCMU, exhibits an identical pH dependence. The half-time for this latter reaction is ca. 4.5 s for the treated membranes and ca. 1.3 s for both control and restored membranes at pH 6.5. However, at pH 7.5 the back reaction has a half-time of ca. 2.9 s in treated membranes and ca. 2.3 s for the restored and control cases.

A stablilization of $Q_A^-$ in treated membranes at pH 6.5, as suggested here, might be achieved by $Q_A^-$ becoming protonated and/or experiencing a conformational change in its protein environment. This interpretation is not in conflict with the observation that the EPR signal of the iron-quinone in PS II is much larger in $HCO_3^-$-depleted PS II particles at pH 6.0 [7].

Plots of the Chl a fluorescence decays from the pH 6.5 data in Fig. 2, following flashes 3 through 10 of a train of actinic flashes given at 1 Hz, are all superimposed on top of one another (data not shown).

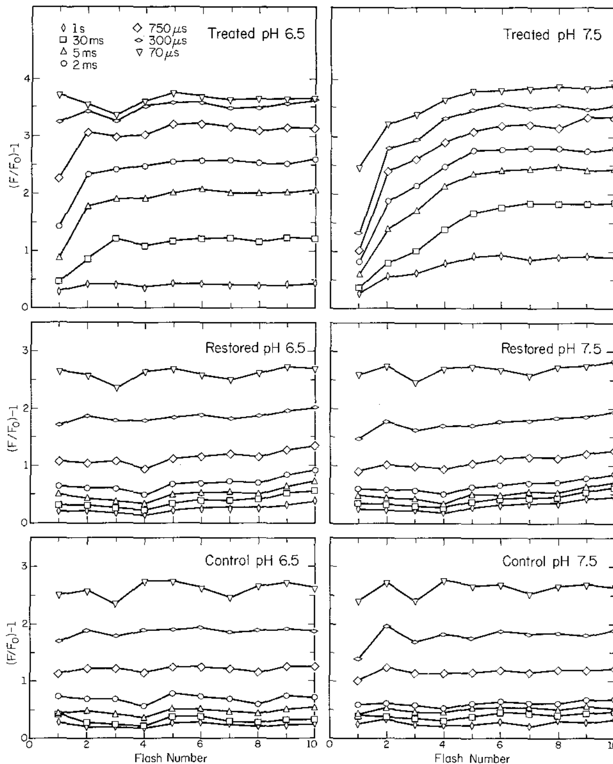


FIGURE 2. Variable Chl a fluorescence yield as a function of flash number showing the $HCO_3^-$-reversible effect in treated membranes at pH 6.5 and pH 7.5. Flash frequency, 1 Hz. The times indicated are when the measuring flash was fired (after Eaton-Rye and Govindjee [8]).

However, for the pH 7.5 data, only the decays for flashes 5 through 10 are superimposed. That is, the fluorescence yield (proportional to $[Q_A^-]$) reaches its maximum after flash 5 at pH 7.5 and after flash 3 at pH 6.5. In addition it can be seen that 2 ms after the actinic flash the maximum fluorescence yield is higher at pH 7.5 (flash 5) than at pH 6.5 (flash 3). It would therefore appear that the successive turnovers of the two-electron gate in treated membranes proceed more slowly at the alkaline pH than at the acidic pH. Since in restored and control membranes the two-electron gate appears to turnover without obvious impediment at both pH 6.5 and 7.5, the above data suggest an induced sensitivity to the availability of protons in the treated membranes, especially at pH 7.5.

Figure 3 demonstrates that even at pH 6.5, the number of turnovers necessary before maximal $[Q_A^-]$ is reached is extended to flash 5 if the flash frequency is increased to 5 Hz. It is therefore possible that a dark-time of 200 ms (at 5 Hz) is insufficient for an equal amount of protonation to occur at the electron acceptor side of PS II than when 1 s (1 Hz) is allowed to elapse between flashes.

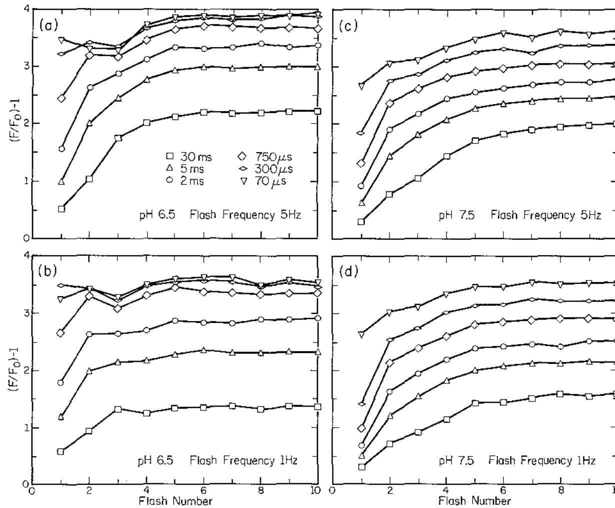


FIGURE 3. Variable Chl a fluorescence yield as a function of flash number at two frequencies (1 or 5 Hz) and at pH 6.5 and 7.5 (after Eaton-Rye and Govindjee [8]).

## 4. SUMMARY

The results here demonstrate, for the first time, that: (1) the effect of anion inhibition/$HCO_3^-$-depletion on the reoxidation of $Q_A^-$ following a single flash is pH dependent with little or no effect observed at pH 7.5, and (2) that in anion inhibited/$HCO_3^-$-depleted membranes, the number of actinic flashes necessary before the maximal $[Q_A^-]$ is observed is dependent on both pH and flash frequency. These observations appear to be consistent with a role for the $HCO_3^-$ anion both in the protonation reactions of the two-electron gate and in maintaining the conformational integrity of PS II for efficient electron transfer.

ACKNOWLEDGEMENT

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