Electron transfer through the quinone acceptor complex of Photosystem II after one or two actinic flashes in bicarbonate-depleted spinach thylakoid membranes

Julian J. Eaton-Rye a,∗ and Govindjee a,b

Departments of a Plant Biology and b Physiology and Biophysics, University of Illinois, Urbana, IL (U.S.A.)

(Received 17 August 1987)
(Revised manuscript received 24 May 1988)

Key words: Anion; Bicarbonate effect; Chlorophyll a fluorescence; Kinetics; Photosystem II; Quinone; (Spinach)

We report here the pH dependence of the kinetics of the decay of variable chlorophyll a fluorescence after one or two actinic flashes in the absence or the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) in HCO3−-depleted or anion-inhibited spinach thylakoid membranes. All the reported effects of HCO3− removal are reversed by the addition of 5 mM HCO3−. The initial first-order component for the oxidation of Qa− (the reduced primary plastoquinone acceptor of Photosystem II (PS II)) by Qb (the secondary plastoquinone acceptor) was reversibly inhibited in a pH-dependent manner in HCO3−-depleted membranes. After a single actinic flash, the half-time of Qa− decay was 630 μs (amplitude, 29%) at pH 6.5 which changed to a value of 320 μs (amplitude, 66%) at pH 7.75. The rate and amplitude at pH 7.75 were approximately the same as found in the restored and control membranes which were pH independent over the same pH range. A similar observation was made after the second actinic flash. Thus, at alkaline pH HCO3−-depleted membranes behave as control membranes with respect to electron flow from QA to Qb or to Qb. The time (t0) at which the [Qa] is 50% of the maximum [Qa] during the back reaction between QA and the S2 state of the oxygen-evolving complex, in the presence of 5 μM DCMU, was increased from 1.3 s in control and restored samples to 5.3 s in HCO3−-depleted samples below pH 7.0, but was unaffected above pH 7.5 (2.3–2.9 s in all cases). Furthermore, a new pathway of QA with a half-time of less than 100 μs was present at pH 6.0 in the presence of DCMU, in approx. one-third of the PS II centers in HCO3−-depleted membranes. The apparent equilibrium for the sharing of an electron between QA and Qb is estimated to decrease by a factor of 4 at pH 6.0 in treated membranes (Kapp ≈ 16) as compared to the restored or control membranes (Kapp ≈ 62); there was no difference in Kapp at pH 7.75. Estimates of the operating redox potential for the Qa/Qb couple from the results presented here indicated that the pH dependence of this parameter is greatly reduced in treated membranes (−60 mV at pH 6.0 to −72 mV at pH 7.75) as compared to restored or control membranes (−25 mV at pH 6.0 to −72 mV at pH 7.75). We discuss our results in the context of a model that envisages HCO3− to act as a proton donor to the protein dissociable group believed to participate in the protonation of Qb. Finally, the possibility of HCO3− being a ligand to Fe2+ in the QA-Fe-Qb complex of the PS II reaction center is also discussed.

∗ Present address: National Institute for Basic Biology, 38 Nishigonaka Myodaijicho, Okazaki, 444 Japan.

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 4-morpholineethanesulfonic acid; Chl, chlorophyll; PS II, Photosystem II; DCMU (dichlor), 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

Correspondence: Govindjee, Department of Plant Biology, University of Illinois at Urbana-Champaign, 289 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801, U.S.A.

0005-2728/88/$03.50 © 1988 Elsevier Science Publishers B.V. (Biomedical Division)
Introduction

The oxidation of QA, the reduced primary plastoquinone acceptor in Photosystem II, is markedly inhibited in membranes depleted of HCO$_3^-$ in the presence of several monovalent anions (see for reviews Refs. 1–3). In particular, formate and nitrite are effective inhibitors, whereas the addition of HCO$_3^-$ is able to restore fully the rate of oxidation of QA to the control value [4–6]. QA is an obligate one-electron acceptor while QB, the secondary plastoquinone acceptor, is reduced to plastoquinol (Q$_{a-}(2H^+)$ or Q$_{aH2}$) by two successive turnovers of the PS II reaction center. QB is readily exchangeable with the plastoquinone (PQ) pool when it is fully oxidized (QB) or reduced (QBH$_2$), while the semiquinone form, Q$_a^-$, exists as a stable bound species [7]. The Q$_a$ binding site is located on the D1 reaction center protein or the herbicide binding protein (see for reviews Refs. 8 and 9). Thus, on consecutive excitations of the PS II reaction center, QA is oxidized by either plastoquinone or plastoquinol at the QA-site (see for a review Ref. 10).

Both the forward rate constants for QA oxidation by QB or QA are decreased following the depletion of HCO$_3^-$, However, we have recently reported that the extent of the inhibition of QA oxidation exhibits a dependency on both the actinic flash frequency and pH [11,12]. Specifically, we observed that the kinetics of QA oxidation after one or two actinic flashes in HCO$_3^-$-depleted membranes were faster at pH 7.5 than pH 6.5. Upon further turnovers of the reaction centers at frequency of 1 Hz this pH dependency was reversed: the oxidation of QA became more inhibited at alkaline pH. Furthermore, at a flash frequency of 5 Hz, the extent of the inhibition observed at pH 6.5 approached that measured for pH 7.5 after the 4th or the 5th actinic flash. To explain these results we proposed a working hypothesis where HCO$_3^-$ protonated the dissociable protein group thought to participate in QB protonation [12]. However, we could not discount the possibility that HCO$_3^-$ also participated in the second protonation step associated with plastoquinol formation.

In the present paper we analyze in detail the kinetics of QA oxidation, at several pHs, after one (by QA) or two (by Q$_a^-$) actinic flashes in the absence or the presence of DCMU (3-(3,4-dichlorophenyl)-1,1-dimethyl urea). Results, reported here, are consistent with our current working hypothesis that HCO$_3^-$ may act as a proton donor group for the protonation of QA and allow us to extend our knowledge about the mechanism of HCO$_3^-$ action in PS II.

Materials and Methods

Thylakoid membranes were prepared from market 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 in the presence of sodium formate. Detailed methods and references for these procedures are given in Ref. 12. The treatment buffer contained CO$_2$-free 300 mM sorbitol, 25 mM sodium formate, 10 mM NaCl, 5 mM MgCl$_2$ and 10 mM sodium phosphate (pH 6.0). The chlorophyll concentration was 250 $\mu$M. The reaction medium contained CO$_2$-free 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 $\mu$M methyl viologen and 0.1 $\mu$M gramicidin. All measurements were made on a sample diluted to contain 5 $\mu$M Chl in a final volume of 100 ml in a dark stirred vat. A flow cuvette was filled from the vat by computer control.

Restored membranes were obtained by adding 5 mM HCO$_3^-$ to a 2 ml aliquot of the treated membrane 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 7.5.

The kinetics of decay of variable Chl a fluorescence at 685 nm (indicating oxidation of QA by either QB or Q$_a^-$) were measured by a weak xenon flash sampling 1% of the reaction centers after each xenon actinic flash. The width at half-peak height for the actinic flash was 2.5 $\mu$s (Ref. 12; also see Refs. 13 and 14).

No significant amount of QA was detected in our preparations following the incubation
HCO₃⁻-depletion treatment when the DCMU-induced variable Chl a fluorescence yield [15] was examined in dark-adapted samples. In addition, our results were unaltered when samples were incubated with 20 μM benzoquinone, a procedure demonstrated to oxidize QA (see, e.g., Ref. 16), prior to the experimental measurement (data not shown). Half-times (t₁/₂) for QA oxidation were obtained by employing the equations derived by Joliot and Joliot [17] relating variable Chl a fluorescence to [QA]. The relationship is written as (see, e.g., Ref. 18):

\[
\frac{F - F_0}{F_m - F_0} = 1 - \frac{1}{p_q} \left( 1 - \left( \frac{1}{p_q} \right)^t \right)
\]

where F is the fluorescence yield at time t, F₀ is the fluorescence yield when all QA is in the oxidized state, Fₘ is the maximum fluorescence yield when all QA is in the reduced state, p, the connection parameter, is taken as the probability of the intersystem energy transfer, and q is the fraction of the closed reaction centers (i.e., q = 1 when QA is maximum). All times labeled as t₅₀, in this paper, are times at which [QA] is 50% of maximum [QA] (at t = 0), whereas all other times are given as half-times (t₁/₂) and presented, together with their amplitudes. These are obtained from plots of log of [QA] as a function of time after evaluation into fast and slow components. Additional details are given in Ref. 12.

Results and Discussion

Oxidation of QA in the absence of an inhibitor

Electron transfer through the PS II plastoquinone acceptors was followed in a step-wise manner by monitoring the decay of variable Chl a fluorescence following one or two actinic flashes. The results of such an experiment, with a dark time of 1 s separating flash 1 and 2, are presented in Figs. 1 and 2.

After a single flash, the time (t₅₀) at which [QA] is 50% of maximum [QA] for the treated membranes is extended from 550 μs to 2.8 ms at pH 6.5 and from 400 μs to 1.5 ms at pH 7.5, but the QA oxidation reaction proceeds to almost the same apparent equilibrium of the restored and control membranes within 100 ms in each case (Fig. 1). Therefore the fraction of QA,QA centers in treated and restored or control membranes when the second flash is spaced 1 s after the first appears unchanged in each instance. The t₅₀ values from Figs. 1 and 2 suggest that the overall rate of QA oxidation is faster at pH 7.5 (1.5 ms (flash 1); 3.6 ms (flash 2)) than pH 6.5 (2.8 ms (flash 1); 11 ms (flash 2)) in treated membranes. This is also true for control and restored membranes after a single flash (460 μs (pH 7.5); 550 μs (pH 6.5)) but little or no effect of pH after a second flash (630 μs (pH 7.5); 600 μs (pH 6.5)) was observed. Bicarbonate depletion causes a greater slowing of QA oxidation by QA (flash 2) than by QA (flash 1) at both pH values. At pH 6.5 the t₅₀ value is extended from 2.8 ms (flash 1) to 11 ms (flash 2) and at pH 7.5 from 1.5 ms (flash 1) to 3.6 ms (flash 2).

To investigate the effects of bicarbonate depletion further, we analyzed semi-logarithmic plots of [QA] against time from the experiments in Figs. 1 and 2. Table 1 summarizes the half-times and amplitudes of the initial first-order decay components revealed. At pH 6.5, it is apparent that after one or two actinic flashes the forward rate constants for these components are decreased (the half-times are increased by an approximate factor of 2) and the corresponding amplitudes decreased. Both effects were found to be reduced at pH 7.5.

In the case of flash 1 the initial first-order component, for restored membranes, is thought to reflect electron transfer from QA to Qₐ in reaction centers which have Qₐ bound before the flash while the remainder of the decay represents transfer to centers which had the Qₐ-site originally unoccupied in the dark [19]. The amplitude of this component is, therefore, a measure of the association constant for plastoquinone that binds to the Qₐ-site. This analysis may apply to control and restored membranes but the interpretation does not readily account for the kinetics observed here for the treated case, although we have considered this possibility earlier [20]. In this instance both the rate and the amplitude are affected. This phenomenon is shown, as a function of pH, for flash 1 and 2 in the experiment in Fig. 2. In both cases, the amplitude and rate for the initial first-order component in treated membranes exhibits a linear
Fig. 1. Decay of variable chlorophyll α fluorescence after a single actinic flash at (a) pH 6.5 and (b) 7.5. F is the Chl α fluorescence yield from the measuring flash with all QA oxidized and F is the yield at the indicated time after the actinic flash. Time is plotted on a logarithmic scale. The insets show the decays of Chl α fluorescence on a linear scale over the first 5 ms.

dependence on pH between 6.5 and 7.75. In treated membranes, at pH 6.5, and after flash 1, we have an amplitude and \( t_{1/2} \) of 29% and 630 μs and at pH 7.75 61% and 240 μs, respectively. In control and restored membranes, at pH 6.5, we have an amplitude and \( t_{1/2} \) of 65% and 320 μs and at pH 7.75 66% and 230 μs. These data indicate little or no pH dependence in the restored case. However, below pH 6.5, the amplitude for the initial rate of Q\( \alpha \) oxidation does appear to be pH dependent in control and restored membranes.

The remaining amplitude, following a single actinic flash, in treated membranes, exhibits an approximate factor of 2 slowing from pH 6.5 (\( t_{1/2} = 5 \) ms) to pH 7.75 (\( t_{1/2} = 9 \) ms). These

<table>
<thead>
<tr>
<th>Flash 1</th>
<th>pH 6.5</th>
<th>pH 7.5</th>
<th>pH 6.5</th>
<th>pH 7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>for treated membranes</td>
<td>350</td>
<td>460</td>
<td>30</td>
<td>54</td>
</tr>
<tr>
<td>for restored membranes</td>
<td>285</td>
<td>250</td>
<td>70</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Flash 2</th>
<th>pH 6.5</th>
<th>pH 7.5</th>
<th>pH 6.5</th>
<th>pH 7.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>for treated membranes</td>
<td>665</td>
<td>465</td>
<td>21</td>
<td>37</td>
</tr>
<tr>
<td>for restored membranes</td>
<td>330</td>
<td>330</td>
<td>75</td>
<td>70</td>
</tr>
</tbody>
</table>
Fig. 2. Decay of variable chlorophyll a fluorescence at (a) pH 6.5 and (b) 7.5 after two actinic flashes spaced at 1 s. Other details are as in the legend of Fig. 1.

kinetics, however, are expected to incorporate a contribution from the Q_B association constant and possibly further complexity arising from PS II heterogeneity [21]; also see Ref. 12. Slow components in the 0.1–10 s range have been routinely observed [22,23].

Semi-logarithmic plots for Q_A oxidation after two actinic flashes have been reported to be relatively independent of pH below the pK, estimated as 7.9 [24], for a dissociable protein group associated with Q_A protonation [19,24]. This rate then slows above the pK. Our results in Fig. 3d) appear consistent with this observation. Here, slowing of Q_A oxidation above pH 7.5 is observed in control, restored and treated membranes. We conclude from the pH dependence (Fig. 3) of the initial first-order decay, after one or two actinic flashes, that treated membranes tend to behave as control and restored membranes at alkaline pH.

The remaining amplitude, after two actinic flashes, in treated membranes, had a t_{1/2} of 14 ms at pH 6.5 which was slowed to 21 ms at pH 7.75 in the experiment shown in Fig. 3. Again, as with the slower phase after the first flash, this decay contains additional contributions from kinetic components in the 0.1–10 s range.

Previous data on the HCO_3^- effect support the conclusion that the PS II plastoquinone acceptor complex undergoes a conformational change in treated membranes (see, e.g., Refs. 25–27). We
suggest from our data in Figs. 1–3 that this change is pH dependent, the extent of the inhibition increasing with decreasing pH. We have suggested that one consequence of this is an inhibition of the protonation steps associated with plastoquinol formation (Ref. 12 and see Refs. 20 and 28).

In brief, our data show that, after one or two actinic flashes, the amplitude and rate of the initial first-order component of Q$_A^-$ oxidation show very little dependence on pH in HCO$_3^-$ restored and control membranes. This is in agreement with earlier published work [19,24]. In contrast, we find that both the rate and amplitude of the initial first-order component of Q$_A^-$ oxidation are pH dependent in treated membranes. We ascribe this behavior to a pH-dependent conformational change on the D1 and/or D2 reaction center proteins (for a discussion of D1 and D2, see Ref. 8) such that at alkaline pH, particularly after a single turnover, the treated membranes tend to behave in a fashion similar to that in the restored and control samples.

However, in treated membranes, in Fig. 3(c) the amplitude for the initial first-order component, after flash 2, does not recover to the same extent as observed after a single flash at alkaline pH. This would be consistent with Q$_B^-$ remaining unprotonated in a significant fraction of centers even though 1 s elapsed between the two actinic flashes. Although this interpretation is speculative, evidence to support a role for HCO$_3^-$ in the protonation of Q$_B^-$ is presented below.

**Oxidation of Q$_A^-$ in the presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea**

The back-reaction of Q$_A^-$ with the S$_2$ state (for a discussion of the S-states, see Ref. 29) of the oxygen-evolving complex, in the presence of 5 mM
DCMU, is seen in Fig. 4 to exhibit a similar pH dependence to that seen for the forward reaction in Figs. 1–3. The times (t₅₀), at which [Qₐ] is 50% of the maximum [Qₐ⁺], for the S₂Qₐ⁻ back reaction were 5.2 s for the treated membranes and 1.3 s for both restored and control membranes at pH 6.5. However, at pH 7.5 this back reaction has a t₅₀ value of 2.9 s in the treated membranes, 2.3 s for the restored membranes and 2.0 s for the control. The plot (Fig. 5) of the reciprocal t₅₀ values for Qₐ⁻ oxidation against pH (6.0–8.0) shows good agreement with that obtained in untreated pea thylakoids [24]. From pH 6.0 to 6.75 the t₅₀ value for the S₂Qₐ⁻ back reaction in treated membranes remains pH independent but a transition to a pH-dependent portion of the curve is observed at pH 7.0. The treated and restored samples give identical results at alkaline pH, the differences only being observed below pH 7.5. The slight difference between the control and restored (or treated) point at pH 7.75 is due to the low r.p.I treatment of the latter samples to achieve the HCO₃⁻ reversible inhibition.

The data point for the treated (HCO₃⁻ depleted) sample at pH 8.0 was omitted in Fig. 5 because of the existence of a fast decay component. At pH 8.0 the treated membranes exhibit a rapid oxidation of Qₐ⁻ with an apparent t₁/₂ of less than 100 µs which cannot be resolved with our instrumentaton. Fig. 6(a) shows that this can be reversed by the prior addition of HCO₃⁻, and Fig. 6(b) demonstrates that this phenomenon is not seen when a second flash is given, in this instance, 1 s after the preceding flash. The electron acceptor responsible for this phenomenon is not known.

The apparent equilibrium constant (K_app) for sharing an electron between QA and QB is given by Eqn. 2 (see Ref. 19)

\[
K_{app} = \frac{[Q_{a}Q_{b}]+[Q_{a}Q_{b}(H^{+})]}{[Q_{a}]+[Q_{a}Q_{a}]} \tag{2}
\]

K_app can be measured from the ratio of the apparent half-times for the back reaction with S₂ in uninhibited compared to DCMU-inhibited centers [14]. Chl a fluorescence measurements to determine the back reaction in uninhibited centers...
require quantitation of the characteristic flash pattern observed due to the differential kinetics of $Q_A^-$ oxidation by either $Q_B$ or $Q_A^-$ [14]. This approach was not possible here, since the treated membranes exhibit an extremely distorted flash pattern and the restored and control cases exhibit a damped oscillation due to the necessary incubation involved in these experiments [12]. However, Vermaas et al. [30] have shown that the back reaction, as measured by $O_2$ evolution kinetics, in the absence of DCMU at pH 6.0 is of the order of 100 s and insensitive to $HCO_3^-$ depletion or an anion inhibitory treatment. This value for the uninhibited back reaction, and the value for the back reaction in the DCMU-inhibited case in Fig. 5, allow us to calculate a value for $K_{app}$ of 16 in the treated membranes and 63 in the restored and control samples. The control value is in agreement with that obtained by Robinson and Corfits [24]. Our data indicate that the apparent equilibrium for the sharing of an electron between $Q_A$ and $Q_B$ experiences a 4-fold shift toward $Q_A^-$ at pH 6.0. This value is a factor of 2 larger than that reported by Vermaas et al. [30]. The difference may partially lie in the fact that the latter authors had used the decay of the uncorrected Chl a fluorescence rather than that of $Q_A^-$ oxidation to estimate $K_{app}$.

The midpoint potential of the $Q_A^-/Q_A^-$ couple has been shown to be unaffected by $HCO_3^-$ depletion [25]. Thus, it is possible to estimate the operational redox potential for the $Q_B^-/Q_B^-$ couple from the following relationship:

$$E_m(Q_B^-/Q_B^-) - E_m(Q_A^-/Q_A^-) = \frac{RT \ln K_{app}}{F}$$

where $R$ is the gas constant, $F$ is the Faraday constant and $T$ is the absolute temperature. Using the value of $-130$ mV (see Ref. 31) for the working redox potential ($E_m$) of $Q_A^-/Q_A^-$, and assuming this to be unchanged in treated membranes, our estimates of $K_{app}$ (see above) give a value of $-25$ mV for the $E_m$ of $Q_B^-/Q_B^-$ couple at pH 6.0 in restored and control membranes and $-60$ mV in the treated membranes. In control pea thylakoids [24] an identical value of $-25$ mV was obtained at pH 6.0 and a value of $-77$ mV at pH 7.75. If we assume that the back reaction of $Q_A^-$ with $Q_S$ at alkaline pH is also unaffected by $HCO_3^-$ depletion, our data in Fig. 5 then suggests a more than 50 mV shift in the operating redox potential of the $Q_A^-/Q_B^-$ couple in restored and control membranes, between pH 7.75 and pH 6.0, while only an approx. 17 mV shift is evident in the treated samples over the same pH range.

The above estimates support the notion that the protonation of the dissociable protein group associated with $Q_B^-$ reduction is reduced in treated membranes. In addition, estimation of the redox midpoint potential for the $Q_B^-/Q_B^-$ couple (see Ref. 19) from the above values suggested only a minor shift in the equilibrium constant for the transfer of an electron from $Q_B^-$ to $Q_B^-$ in treated membranes. However, further experimental studies are required before a conclusion can be drawn regarding the participation of $HCO_3^-$ in the second protonation step accompanying this reaction.

**Bicarbonate binding**

Although our data does not address the question of the site of $HCO_3^-$ binding in PS II, there are several lines of evidence to support the hypothesis that $HCO_3^-$ is a ligand to iron in the $Q_A^-Fe-Q_B$ complex of PS II.
(a) The iron in the reaction center of the photosynthetic bacterium *Rhodopseudomonas viridis* is liganded to four histidines and to a glutamate. In PS II this glutamate is not present on D2 and HCO$_3^-$ has been suggested to serve as the ligand in its place [32]. In addition, no HCO$_3^-$-reversible anionic inhibition was observed on quinone-mediated electron transfer in the reaction center preparations from another bacterium *Rhodospirillum rubrum* (Shopes, R.J., Blubaugh, D. and Govindjee, unpublished observations).

(b) In PS II particles, prepared from spinach, the $Q_A^{-}$-Fe$^{2+}$ EPR signal at $g = 1.82$ increased 10–12-fold upon HCO$_3^-$ removal in the presence of formate [26]. No such effect was observed in chromatophores from *R. rubrum* [33,34].

(c) The redox midpoint potential of the $Q_A^{-}/Q_A$ couple is unaffected by HCO$_3^-$ depletion [25]. This may suggest that HCO$_3^-$ does not bind directly to $Q_A$.

(d) The Fe$^{2+}$/Fe$^{3+}$ couple has been identified as the $Q_A/Q_A^{-}$ couple by Petrouleas and Diner [35]. Oxidation of this couple by exogenous oxidants in the presence of DCMU is dependent on a strict order of addition. Addition of DCMU prior to the exogenous oxidant prevents Fe$^{3+}$ or $Q_A^{-00}$ oxidation [35–37]. Complete reversal of the inhibition seen by HCO$_3^-$ depletion in the presence of formate is only possible in Fig. 5 when HCO$_3^-$ is added before the DCMU (see also Ref. 40).

(e) The oxidation of Fe$^{2+}$ to Fe$^{3+}$ by high potential quinones is blocked in the presence of formate [39].

**Conclusion**

HCO$_3^-$ depletion in the presence of inhibitory anions inhibits the oxidation of $Q_A^{-}$ in PS II reaction centers [4–6]. The mechanism for this phenomenon is not yet understood. In this paper, we have presented new observations on the pH dependence of $Q_A^{-}$ oxidation after one or two actinic flashes in treated membranes. Between pH 6.8 and pH 7.75 our results show that the rate and amplitude of the initial first-order component of the kinetics of $Q_A^{-}$ oxidation are pH dependent. The oxidation is slowed at acidic pH, but resembles that in the control at basic pH. A similar, although quantitatively different, pH dependence was observed for the slow $Q_A^{-}$ oxidation, by a back reaction with the S$_2$ state, in the presence of diuron (DCMU). From these results it appears that the equilibrium constant for $Q_A^{-}Q_B^{-} \rightleftarrows Q_AQ_B^{-}$ is almost pH independent in treated membranes.

In contrast, we found that both the rate and amplitude for these reactions were independent of pH across the same pH range in restored and control membranes. In addition, the equilibrium for $Q_A^{-}Q_B^{-} \rightleftarrows Q_AQ_B^{-}$ was pH dependent. These results are in agreement with other published work [19,24].

We suggest that replacement of HCO$_3^-$ by HCO$_2^-$ introduces a conformational change in the PS II quinone acceptor complex that is pH dependent. In addition, the protonation of $Q_B^{-}$ may be inhibited as a consequence of the conformational change or because HCO$_3^-$ has been replaced by formate. In our working model HCO$_3^-$ is suggested to be a ligand to Fe$^{2+}$ while the hydroxyl group of the bound HCO$_3^-$ (also see Refs. 38 and 42) protonates a dissociable protein group that is functional in the protonation of $Q_B^{-}$ (also see Refs. 19 and 41). Formate lacks such a hydroxyl group that could provide a proton. Furthermore, we do not discount the possibility that HCO$_3^-$ may participate in the second protonation step associated with plastoquinol formation or that more than one HCO$_3^-$ may be bound per PS II [42].

We have suggested that the rate-limiting step of photosynthetic electron transport, in treated membranes, may be at the level of the protonation steps accompanying plastoquinol formation (see Ref. 12).

**Acknowledgements**

We are grateful to Drs. A.R. Crofts and H.H. Robinson and our reviewers for helpful comments. This work was supported by a NSF grant (PCM 83-06061) to Govindjee. We are indebted to Dr. Don Ort for encouragement and support during this research.

**References**
