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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.)

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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 HCO_3^--depleted or anion-inhibited spinach thylakoid membranes. All the reported effects of HCO_3^- removal are reversed by the addition of 5 mM HCO_3^-. The initial first-order component for the oxidation of Q_A^- (the reduced primary plastoquinone acceptor of Photosystem II (PS II)) by Q_B (the secondary plastoquinone acceptor) was reversibly inhibited in a pH-dependent manner in HCO_3^--depleted membranes. After a single actinic flash, the half-time of Q_A^- 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 HCO_3^--depleted membranes behave as control membranes with respect to electron flow from Q_A to Q_B or to Q_B^-. The time (t_{50}) at which the $[Q_A^-]$ is 50% of the maximum $[Q_A^-]$ during the back reaction between Q_A^- and the S_2 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 HCO_3^--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 Q_A^- with a half-time of less than 100 µs was present at pH 8.0 in the presence of DCMU, in approx. one-third of the PS II centers in HCO_3^--depleted membranes. The apparent equilibrium for the sharing of an electron between Q_A and Q_B is estimated to decrease by a factor of 4 at pH 6.0 in treated membranes ($K_{app} \approx 16$) as compared to the restored or control membranes ($K_{app} \approx 62$); there was no difference in K_{app} at pH 7.75. Estimates of the operating redox potential for the Q_B/Q_B^- 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 HCO_3^- to act as a proton donor to the protein dissociable group believed to participate in the protonation of Q_B^-. Finally, the possibility of HCO_3^- being a ligand to Fe^{2+} in the Q_A-Fe-Q_B 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-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid; Chl, chlorophyll; PS II, Photosystem II; DCMU (diuron), 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.

Introduction

The oxidation of Q_A^-, 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 Q_A^- to the control value [4–6].

Q_A is an obligate one-electron acceptor while Q_B, the secondary plastoquinone acceptor, is reduced to plastoquinol ($Q_B^{2-}(2H^+)$ or Q_BH_2) by two successive turnovers of the PS II reaction center. Q_B is readily exchangeable with the plastoquinone (PQ) pool when it is fully oxidized (Q_B) or reduced (Q_BH_2), while the semiquinone form, Q_B^-, exists as a stable bound species [7]. The Q_B 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, Q_A^- is oxidized by either plastoquinone or plastosemiquinone at the Q_B-site (see for a review Ref. 10).

Both the forward rate constants for Q_A^- oxidation by Q_B or Q_B^- are decreased following the depletion of HCO_3^-. However, we have recently reported that the extent of the inhibition of Q_A^- oxidation exhibits a dependency on both the actinic flash frequency and pH [11,12]. Specifically, we observed that the kinetics of Q_A^- 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 Q_A^- 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 Q_B^- 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 Q_A^- oxidation, at several pHs, after one (by Q_B) or two (by Q_B^-) 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 Q_B^-, 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 µM. 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 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 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 Q_A^- by either Q_B or Q_B^-) 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 µs (Ref. 12; also see Refs. 13 and 14).

No significant amount of Q_B^- was detected in our preparations following the incubation or

HCO_3^--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 Q_B^- (see, e.g., Ref. 16), prior to the experimental measurement (data not shown). Half-times ($t_{1/2}$) for Q_A^- oxidation were obtained by employing the equations derived by Joliot and Joliot [17] relating variable Chl *a* fluorescence to $[Q_A^-]$. The relationship is written as (see, e.g., Ref. 18):

$$\frac{F-F_0}{F_m-F_0}=\frac{1-p}{1-pq} \tag{1}$$

where F is the fluorescence yield at time t, F_0 is the fluorescence yield when all Q_A is in the oxidized state, F_m is the maximum fluorescence yield when all Q_A 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 Q_A^- is maximum). All times labeled as t_{50}, in this paper, are times at which $[Q_A^-]$ is 50% of maximum $[Q_A^-]$ (at $t=0$), whereas all other times are given as half-times ($t_{1/2}$) and presented, together with their amplitudes. These are obtained from plots of log of $[Q_A^-]$ as a function of time after evaluation into fast and slow components. Additional details are given in Ref. 12.

Results and Discussion

Oxidation of Q_A^- 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 (t_{50}) at which $[Q_A^-]$ is 50% of maximum $[Q_A^-]$ 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 Q_A^- 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 $Q_AQ_B^-$ 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 t_{50} values from Figs. 1 and 2 suggest that the overall rate of Q_A^- 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 Q_A^- oxidation by Q_B^- (flash 2) than by Q_B (flash 1) at both pH values. At pH 6.5 the t_{50} 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 $[Q_A^-]$ against time from the experiments in Figs. 1 and 2. Table I 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 Q_A^- to Q_B in reaction centers which have Q_B bound before the flash while the remainder of the decay represents transfer to centers which had the Q_B-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 Q_B-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 liner

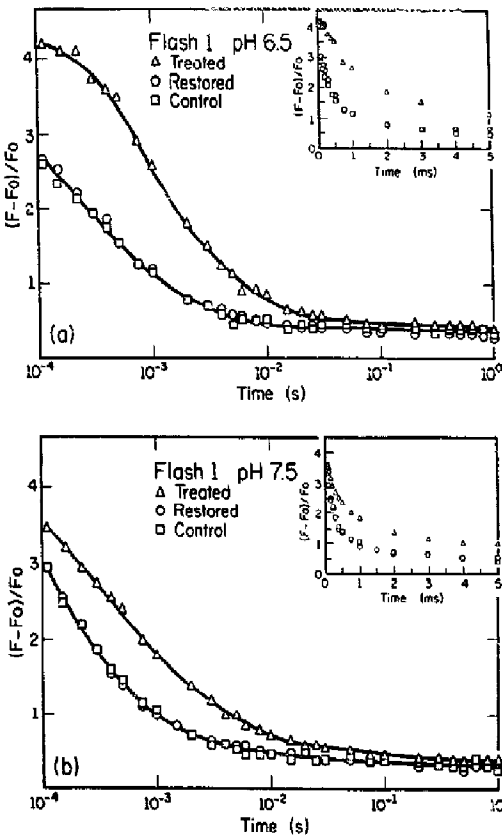

Fig. 1. Decay of variable chlorophyll *a* fluorescence after a single actinic flash at (a) pH 6.5 and (b) 7.5. F_0 is the Chl *a* fluorescence yield from the measuring flash with all Q_A oxidized and F is the yield at the indicated time after the actinic flash. Time is plotted on a logarithmic scale. The insets show the decays of Chl *a* 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_A^- 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 I

HALF-TIMES AND CORRESPONDING AMPLITUDES OF THE INITIAL FIRST-ORDER COMPONENT OF Q_A^- OXIDATION FROM THE EXPERIMENT SHOWN IN FIGS. 1 AND 2 FOR TREATED AND RESTORED MEMBRANES AT pH 6.5 AND 7.5 AFTER ONE OR TWO ACTINIC FLASHES SPACED AT 1 s

	Half-time (µs)		Amplitude (%)	
	pH 6.5	pH 7.5	pH 6.5	pH 7.5
Flash 1				
for treated membranes	550	460	30	54
for restored membranes	285	250	70	71
Flash 2				
for treated membranes	665	465	21	37
for restored membranes	330	330	75	70

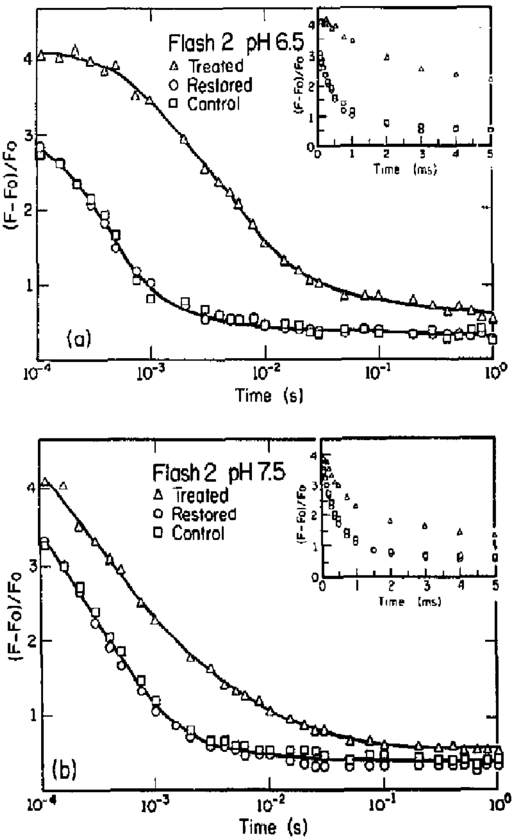

Fig. 2. Decay of variable chlorophyll *a* fluorescence at (a) pH 6.5 and (b) 7.5 after two actinic flashes spaced at 1 s. Other details are as in the legend of Fig. 1.

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 p*K*, estimated as 7.9 [24], for a dissociable protein group associated with Q_B^- protonation [19,24]. This rate then slows above the p*K*. 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

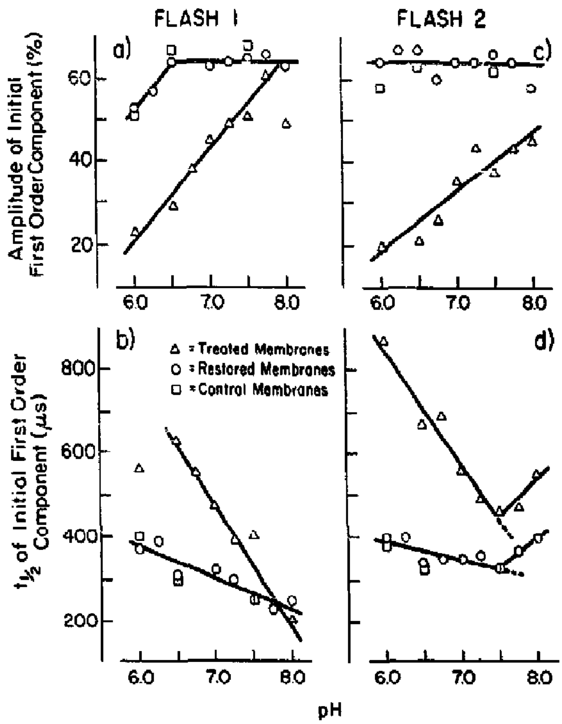

Fig. 3. Plot of the amplitudes and half-times for the initial first-order component of Q_A^- oxidation as a function of pH after 1 and 2 flashes. In a) the data are for the amplitude after a single actinic flash and in b) for the rate after a single flash. In c) and d), the data are for the amplitude and rate respectively following two actinic flashes spaced at 1 s. The lines are drawn through the data for the treated and restored membranes only.

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 μM

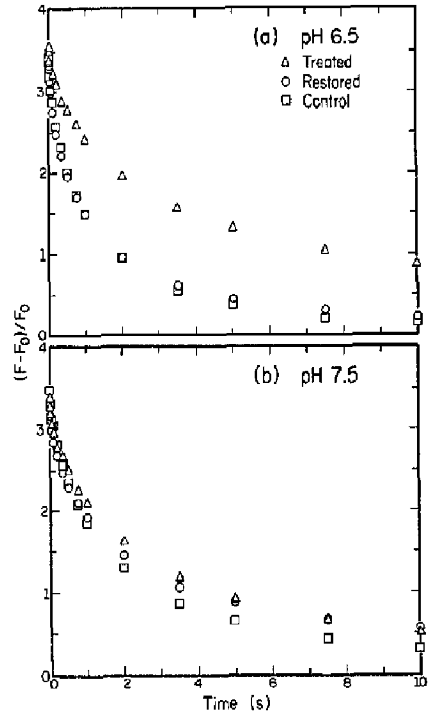

Fig. 4. Decay of variable chlorophyll *a* fluorescence after a single actinic flash at (a) pH 6.5 and (b) pH 7.5 in the presence of 5 μM diuron (DCMU). F_0 is the Chl *a* fluorescence yield from the measuring flash with all Q_A oxidized and F is the yield at the indicated time after the actinic flash.

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 (t_{50}), at which $[Q_A^-]$ is 50% of the maximum $[Q_A^-]$, for the $S_2Q_A^-$ 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 t_{50} 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 t_{50} values for Q_A^- 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 t_{50} value for the $S_2Q_A^-$ 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 pH treatment of the latter samples to achieve the HCO_3^--reversible inhibition.

The data point for the treated (HCO_3^--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 Q_A^- with an apparent $t_{1/2}$ 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 HCO_3^-, 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 Q_A and Q_B is given by Eqn. 2 (see Ref. 19)

$$K_{app} = \frac{[Q_AQ_B^-] + [Q_AQ_B^-(H^+)]}{[Q_A^-] + [Q_A^-Q_B]} \tag{2}$$

K_{app} can be measured from the ratio of the apparent half-times for the back reaction with S_2 in uninhibited compared to DCMU-inhibited centers [14]. Chl *a* fluorescence measurements to determine the back reaction in uninhibited centers

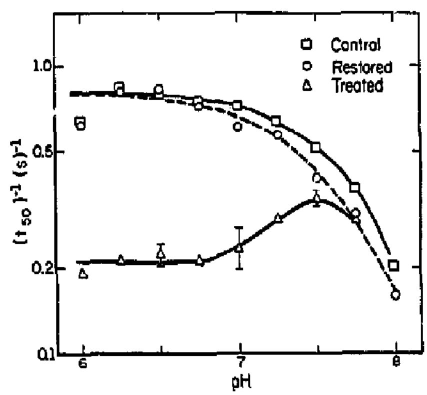

Fig. 5. The reciprocal of the time (t_{50}) at which $[Q_A^-]$ is 50% of the maximum $[Q_A^-]$ in the presence of 5 μM diuron (DCMU) plotted as a function of pH. For additional details, see text.

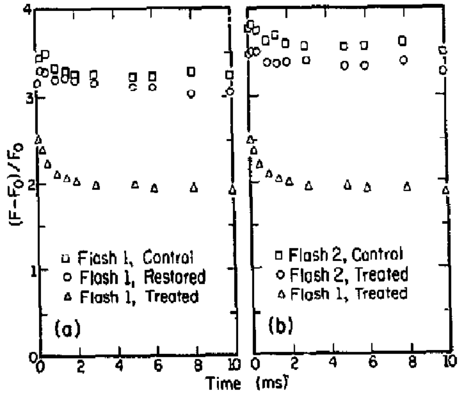

Fig. 6. Decay of variable chlorophyll *a* fluorescence at pH 8.0 in the presence of 5 μM diuron (DCMU). Other details are as in Fig. 4. In (a) the decay after a single flash is shown for treated, restored and control membranes. In (b) the decay after two actinic flashes, spaced at 1 s, is shown for control and treated membranes. The decay for the treated sample after one actinic flash is also shown for comparison.

require quantitation of the characteristic flash pattern observed due to the differential kinetics of Q_A^- oxidation by either Q_B or Q_B^- [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 Corfts [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:

$$E_m(Q_B/Q_B^-) - E_m(Q_A/Q_A^-) = \frac{RT \ln K_{app}}{F} \quad (3)$$

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_B^- with S_2 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_B/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^{2-} 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_BH_2 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_{400}/Q_{400}^+ 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^{2+} or Q_{400} 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 6.5 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 $Q_A^-Q_B \rightleftharpoons Q_AQ_B^-$ 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 $Q_A^-Q_B \rightleftharpoons Q_AQ_B^-$ 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).

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