The Effects of Bicarbonate Depletion and Formate Incubation on the Kinetics of Oxidation-Reduction Reactions of the Photosystem II Quinone Acceptor Complex

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Chloroplast thylakoid membranes depleted of bicarbonate exhibit a slowed oxidation of the primary quinone acceptor (Q_A) by the secondary quinone acceptor (Q_B) of photosystem II. The kinetics of these slowed reactions have been followed by using short xenon flashes of light both to excite photosystem II and to probe the redox state of Q_A. Thylakoids incubated with formate but not depleted of bicarbonate showed the same pattern of slowed reaction kinetics of the quinone acceptors as seen in bicarbonate-depleted thylakoids. This led us to conclude that there was a simple competition between bicarbonate and formate at this site; however, steady-state electron transfer measured with an oxygen electrode showed that although the bicarbonate-depleted thylakoids were indeed inhibited, rates in the formate-incubated thylakoids were only slightly slowed. We suggest that the inhibition seen at the quinone acceptor site of photosystem II depends in a subtle way upon the rate of exchange of bicarbonate and formate at this site.

Introduction

Good [1] showed that steady-state electron transport in isolated chloroplasts was strongly inhibited by bicarbonate depletion when high concentrations of formate (or acetate) were present. More recent work [2–6] has localized one of the sites interfered with by the formate-inhibition/bicarbonate-depletion as the secondary quinone acceptor site (Q_B) of photosystem II (PS II). Current thinking is that Q_B is a binding site where plastoquinone and plastoquinol from the thylakoid membrane associated plastoquinone pool are in exchange [7]. This site functions as a two-electron gate; electrons arrive singly from the obligate one-electron donor Q_A and are either stabilized at the Q_B site as plastosemiquinone or leave as the two-electron reduced species, plastoquinol, if the center had plastosemiquinone anion already present [8–10]. The decay of Q_A^- can be indirectly followed by putting all centers in a highly-fluorescent closed state (Q_A^-) with an actinic flash, then measuring the fluorescence yield with weak flashes at specified intervals. After a delay, some centers become weakly-fluorescent open centers, due to the oxidation of Q_A^-. We have employed this technique (as have others [2, 5]) to try to separate the effects of formate on the functioning of this site from the effects of bicarbonate depletion.

Abbreviations: PS II, photosystem II; Q_A, primary quinone acceptor of PS II; Q_B, secondary quinone acceptor of PS II; Chl, chlorophyll.

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Materials and Methods

Pea thylakoid suspensions were prepared as described earlier [11]; the preparation was modified by the addition of benzoquinone (20 μM) to oxidize Q_B^- in all PS II acceptor complexes [12]. These thylakoids were used directly (control), or subjected to either bicarbonate depletion or formate incubation. For bicarbonate depletion, thylakoids at 125 μg Chl/ml were stirred in the dark for one hour with the depletion medium (300 mM sorbitol, 25 mM sodium formate, 10 mM phosphate (pH = 5.8), 10 mM NaCl, 5 mM $MgCl_2$, equilibrated with an atmosphere of only N_2). For formate incubation, thylakoids at 125 μg Chl/ml were stirred in the dark for one hour with the reaction medium (300 mM sorbitol, 25 mM sodium formate, 25 mM phosphate

buffer (pH = 6.5), 10 mM NaCl, 5 mM $MgCl_2$, equilibrated with an atmosphere of O_2/N_2 with 390 ppm CO_2). For the fluorescence experiments, control, bicarbonate-depleted, or formate-incubated samples were diluted in a dark vat to 5 μg Chl/ml in 50 ml of the reaction medium. For the bicarbonate-depleted experiments, the above reaction medium was equilibrated with CO_2-free air; for the control experiments, the medium contained no formate. The same preparations were assayed for O_2 evolution in a Clark-type oxygen electrode in saturating orange light. The kinetics of decay of variable chlorophyll *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 as previously described [11].

Results and Discussion

There has been considerable controversy over the role of bicarbonate in the control of PS II and the part played by formate, which has also been present in most bicarbonate depletion experiments. Possibly formate itself contributes to the inhibition seen when bicarbonate is depleted [5, 13–15]. To investigate this problem, we have measured the kinetics of the reactions of the two-electron gate of PS II of thylakoids depleted of bicarbonate or incubated with formate. For formate treatment, we incubated thylakoid suspensions for one hour in the reaction medium commonly used in measurements of bicarbonate-depleted thylakoids. However, the thylakoids had not been previously depleted of bicarbonate, and the reaction medium was kept in equilibrium with normal atmospheric air (with 390 ppm CO_2). Thylakoids incubated with formate in this way were compared to those that had been depleted of bicarbonate, and measured in a depleted reaction medium containing formate. Figs. 1 and 2 show that the decay of variable fluorescence (indicating oxidation of Q_A^-) after one or three actinic flashes was nearly identical with either formate-incubated or bicarbonate-depleted samples. The decays indicate considerable slowing of the oxidation of Q_A^-. The decay of variable fluorescence for both preparations after flash two was intermediate between flash one and flash three, and after more than three flashes, the pattern of flash three was repeated (data not shown). With both preparations, the addition of 10 mM bicarbonate restored

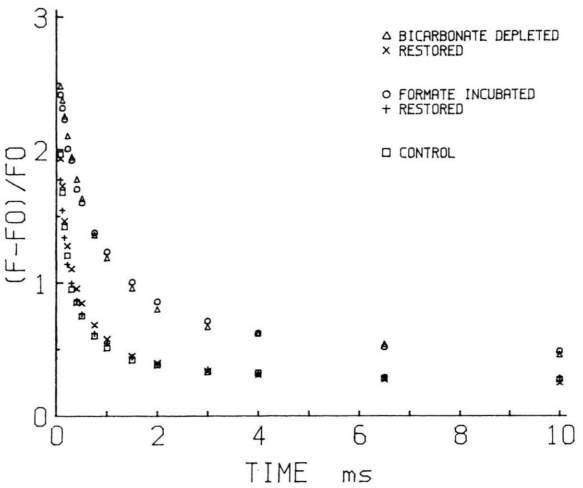

Fig. 1. Decay of variable fluorescence after one actinic flash. Restored decays were after the addition of 10 mM bicarbonate. FO is the chlorophyll *a* fluorescence yield from the measuring flash when all Q_A was oxidized and F was the yield at the indicated time after the actinic flash. The reaction medium (see Materials and Methods) was supplemented with 0.1 mM methylviologen, and 0.1 μM gramicidin. Half-times were determined by correcting the variable fluorescence for quenching [18]. The half-times were: for bicarbonate-depleted, 1.2 ms; for formate-incubated, 1.2 ms; and for control, 230 μs.

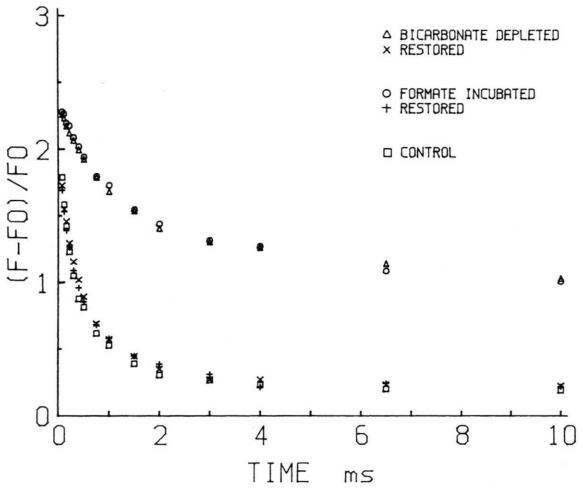

Fig. 2. Decay of variable fluorescence after three actinic flashes spaced at 1 s. Other conditions were as in Fig. 1. The half-times were: for bicarbonate-depleted, 13 ms; for formate-incubated, 10 ms; and for control, 360 μs.

the decays to the pattern seen with control thylakoids (Figs. 1 and 2). These results would suggest that formate and bicarbonate compete for a site at the reducing side of PS II (see *e.g.* [16, 17]): if formate is present at low bicarbonate concentrations, forward

electron flow is slowed; if bicarbonate is present in excess, or at physiological concentrations in the absence of formate, the normal flow is restored. During the formate incubation, the transition from fast to slow decay of variable fluorescence was accelerated under any of the following conditions: a) the concentration of formate was increased; b) the pH was lowered; or c) when the CO_2 concentration was lowered (data not shown). All these observations support the above hypothesis, and suggest further that the equilibria of the formic acid/formate and carbonic acid/bicarbonate systems may be important in the competition.

We have quoted in the legends to Figs. 1 and 2 the overall half-times for the decays of Q_A^- (after correcting for quenching [18]). The corrected data for the decay after the third flash of the bicarbonate-depleted thylakoids has been analyzed for discrete exponential decay components [19]. The percentage contributions and half-times were: 30%, 760 μs; 26%, 7.4 ms; 27%, 280 ms; 17%, 1.1 s. The overall half-time for the oxidation of Q_A^- after the third of three actinic flashes (at 1 Hz) was 13 ms. This was much faster than the decay after the third flash (at 33 Hz) of 140 ms observed by Govindjee *et al.* [2] (see also [4, 20]). It is probable that the 280 ms decay component measured here corresponds to the decay observed earlier [2], and that as the actinic flashes are spaced closer together, this longer decay component would predominate (see [4]).

Figs. 1 and 2 suggest that in both formate-treated and bicarbonate-depleted preparations the acceptors of PS II were inhibited in an identical fashion. However, measurements on the same preparations of steady-state electron transport (H_2O to ferricyanide) under saturating continuous light, measured with the oxygen electrode, showed that although the bicarbonate-depleted thylakoids were inhibited, the rates with formate-incubated thylakoids were only slightly slowed (Fig. 3). To resolve the paradox presented by these results, we suggest that the key to the formate-inhibition/bicarbonate-restoration of electron flow at the acceptor side of PS II must involve the frequency of excitation of PS II and the dynamic exchange of formate and bicarbonate at the acceptor side of PS II. We plan to investigate the effect of excitation frequency in an attempt to resolve the apparent conundrum presented by these flash-fluorescence and oxygen electrode data.

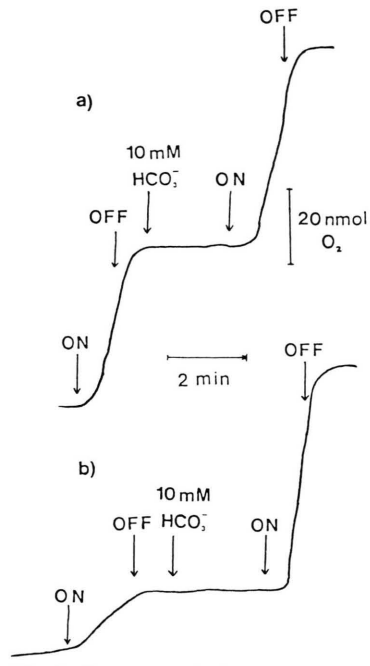

Fig. 3. Oxygen evolution in formate-incubated and bicarbonate-depleted thylakoids. a) Formate-incubated thylakoids (initial rate, in μ eq. (mg Chl)$^{-1}$ h^{-1}, was 516, and after the addition of excess bicarbonate 626); b) bicarbonate-depleted thylakoids (initial rate, in μ eq. (mg Chl)$^{-1}$ h^{-1}, was 63, and after the addition of bicarbonate 698). The reaction medium (see Materials and Methods) was supplemented with 0.5 mM ferricyanide, 10 mM ammonium chloride, 0.01 μM gramicidin. Thylakoid concentration was 20 μg Chl/ml.

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