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A Dual Bicarbonate-Reversible Formate Effect in *Chlamydomonas* Cells

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Summary

It is shown here that a dual bicarbonate-reversible formate inhibition of electron flow exists in the intact cells of a green eukaryotic alga, *Chlamydomonas reinhardtii.* There are two suggested sites of inhibition, one after and the other before Q_A, where Q_A is the bound plastoquinone electron acceptor of Photosystem II (PS II). A long term (hours) formate treatment slows the rate of oxidation of Q_A^-, measured by variable chlorophyll *a* fluorescence decay after an actinic flash. Concomitant with a 2-fold increase in amplitude of the slow component, the amplitude of the fast component decreases at both pH 6.5 and 7.5. The half-times of faster decay increase from 285 μs to 560 μs (pH 6.5) and from 256 μs to 490 μs (pH 7.5), and the slower decay from 37 to 60 ms (pH 6.5) and from 24 to 80 ms (pH 7.5). Addition of 2.5 mM HCO_3^- to the formate-treated samples fully reverses all these effects. The anion effects on the electron flow from Q_A^- to Q_B (where Q_B is the second plastoquinone acceptor) were accompanied by effects on the dimethylquinone/ferricyanide Hill reaction: a 4-fold stimulation was observed upon addition of 20 mM HCO_3^- (pH adjusted, 6.5) to formate-treated samples. Since this stimulation was observed in the presence of 2,5-dibromo-6-isopropyl-p-benzoquinone, which inhibits intersystem electron flow, it is unrelated to CO_2 fixation in intact systems. A short term (minutes) formate treatment reversibly diminishes chlorophyll *a* fluorescence. This effect is present even in mild heat-treated (45 °C, 3 min) and 10 mM hydroxylamine-treated (pH 7.3) cells. Thus, it is suggested that it is between the hydroxylamine donation site (electron donor Z or D) and Q_A.

Key words: Chlamydomonas reinhardtii, bicarbonate reversible formate inhibition, chlorophyll a fluorescence decay, chlorophyll a fluorescence transient, long-term and short-term formate treatment, oxygen evolution.

Abbreviations: Chl = chlorophyll; D = secondary electron donor to Photosystem II; DBMIB = 2,5-dibromo-6-isopropyl-p-benzoquinone; DMQ = dimethylquinone; Q_A = the one-electron acceptor primary bound plastoquinone of Photosystem II; Q_B = the two-electron acceptor secondary plastoquinone of Photosystem II; PS II = photosystem II; Z = the immediate electron donor to Photosystem II reaction center.

Introduction

Bicarbonate was discovered by Warburg and Krippahl (1958) to stimulate electron flow in Hill reaction in chloroplasts. In order to obtain reproducible and large bicarbonate effects, necessary to study the mechanism of this phenomenon, samples are usually pretreated with certain anions (Govindjee and van Rensen 1978), particularly formate (Vermaas and Govindjee 1981). Bicarbonate was shown to stimulate electron transport in the whole chain or Photo-

system II in acetate or formate-treated membranes (Govindjee and van Rensen 1978, Vermaas and Govindjee 1981, Stemler 1985, van Rensen and Snel 1985, Govindjee and Eaton-Rye 1986). Furthermore, it was demonstrated (Govindjee et al. 1976, Jursinic et al. 1976, Eaton-Rye and Govindjee 1988 a, b) that the oxidation of Q_A^-, the reduced form of the first plastoquinone electron acceptor of photosystem II, is dramatically slowed down in formate-treated chloroplasts; subsequent addition of bicarbonate entirely reverses the inhibition obtained after anion treatment. Reoxidation of Q_A^-, as measured by the absorbance change at 320 nm (Siggel et al. 1977, Farineau and Mathis 1983), is calculated to be 10–20 fold faster upon the readdition of HCO_3^- to formate-treated membranes. Thermoluminescence (Govindjee et al. 1984) and chlorophyll (Chl) *a* fluorescence yield measurements (Eaton-Rye and Govindjee 1988 a) after a series of single-turnover saturated light flashes suggest a dramatic inhibition of electron flow after the third and subsequent flashes in formate-treated membranes; this inhibition was relieved by the readdition of bicarbonate. It was earlier suggested (Blubaugh and Govindjee 1988 a) that there may be two major sites of anion effects on the acceptor side of photosystem II in higher plant chloroplasts: one near the Fe between Q_A and Q_B and the other on arginine in the D-1 protein. In view of our suggestion that (a) HCO_3^- is the active species in thylakoids (Blubaugh and Govindjee 1986) and (b) at pH of the stroma (pH 8.0), there is much more HCO_3^- (300 μM) than its Kd (40–60 μM) (Blubaugh and Govindjee 1988 a), formate treatment is suggested to lead to bicarbonate depletion. This notion was challenged by the recent work of Stemler (1989) in which he was unable to observe the release of CO_2, at pH 6.0, upon addition of formate. However, Govindjee, Weger, Turpin,Van Rensen, de Vos and Snel (unpublished, 1989) have observed the release of about 1 CO_2 per reaction center II at pH 6.5. Furthermore, the work of Khanna et al. (1981) suggests that the addition of formate produces a conformational change in the photosystem II quinone acceptor complex and that this is pH dependent.

The above-mentioned bicarbonate-reversible formate effect is absent in photosynthetic bacteria (Shopes et al. 1989) but is present in cyanobacteria (Cao and Govindjee 1988). It has also been observed in non-anion treated intact leaves (Garab et al. 1983, Ireland et al. 1987) and in eukaryotic algal cells (Mende and Wiessner 1985, Govindjee and Eaton-Rye 1986), the latter being an extremely useful model system for studying the molecular mechanism of the bicarbonate reversible anion effect *in vivo*. However, these latter data are limited, and none report the direct effect of the bicarbonate-reversible anion effect on electron flow from Q_A to Q_B observed in isolated thylakoids (Govindjee et al. 1976, Jursinic et al. 1976, Eaton-Rye and Govindjee 1988 a, b) and cyanobacteria (Cao and Govindjee 1988). In order to accentuate this effect and, thus, to use the data for studying the molecular mechanism of the anion effect, we applied the formate treatment methods of higher plant thylakoids to *Chlamydomonas* cells. We present here, for the first time, new data on the existence of the bicarbonate-reversible formate effect on the electron flow from Q_A to Q_B in an eukaryotic cell. Furthermore, we observed the existence of another effect between the hydroxylamine donation site and Q_A. Preliminary abstracts of this work have been presented earlier (El-Shintinawy and Govindjee 1989 a, b).

Materials and Methods

Chlamydomonas reinhardtii cells were grown anaerobically in Tris-Acetate culture medium, (17 mM, pH 7.3, including 1 mM Phosphate, Gorman and Levine 1965) at 25 °C. During growth, the cell culture was placed in 125 mL flasks on a gyratory shaker under continuous illumination from fluorescent lamps (Gorman and Levine 1965). After 4 days, dark green cells were collected for assays. Thylakoid isolation was made as described by Diner and Wollmann (1980). Chlorophyll concentration was determined by the method of MacKinney (1941).

Formate treatment of cells was carried out with a method described by Eaton-Rye and Govindjee (1988 a, b) for higher plant thylakoids, but with the major modification that a diluted culture medium (containing 0.6 mM acetate) was used as a depletion medium after the addition of 25 mM $NaHCO_2$ and lowering the pH to 5.8 to enhance the effect under investigation. For long-term formate treatment, *Chlamydomonas* cells were incubated for 3 h at 20 °C in the above medium under constant flow of N_2 gas over, not in, the medium. The *short-term* formate treatment was carried out by incubating the cells (Chl = 250 μg/mL) in TAP including 25 mM $NaHCO_2$ at pH 5.8 for 5 min. Then the suspension was diluted to 20 μg/mL by adding TAP only without formate at pH 6.5 or 7.5. Bicarbonate-restored samples were prepared by adding 2.5 mM $NaHCO_3$ to the formate-treated samples.

Mild heating was at 45° for 3 min; 10 mM freshly prepared hydroxylamine (pH ajdusted to 7.3) was used, when needed, as an artificial electron donor to PS II. The time of treatment with hydroxylamine was 5 min.

Oxygen evolution rates were determined polarographically using a Yellow Spring Instrument Clark-type electrode. Illumination was provided by a Kodak Carousel 4200 slide projector equipped with a Corning CS 3-68 yellow filter. One mM 2,5-dimethyl-p-benzoquinone (DMQ) was used as an artificial electron acceptor, 1 mM ferricyanide was used to keep DMQ in the oxidized state and 0.5 μM 2,5-dibromo-6-isopropyl-p-benzoquinone (DBMIB) was used as an inhibitor of electron flow between photosystem II and I (Trebst et al. 1970). *Chlamydomonas* cell suspension containing 20 μg Chl/mL was used for the oxygen evolution measurements.

Chlorophyll (Chl) *a* fluorescence transient measurements were made by using a home-built spectrofluorometer. The exciting light was provided by a Kodak 4200 projector with the light filtered by two Corning filters (a blue CS 4-76 and a yellow CS 3-73). Fluorescence emission was detected, with a slit width of 3.3 nm, by a S-20 photomultiplier (EMI 9558B) through a Bausch and Lomb monochromator protected from the exciting light by a red Corning CS 2-61 filter. The photon flux density at the sample was 40 μmol $m^{-2}s^{-1}$. Signals were stored and analyzed by a Biomation 805 waveform recorder and a LSI-11 computer (Blubaugh and Govindjee 1988 b).

The kinetics of decay of Q_A^- to Q_A was obtained through measurements on Chl *a* fluorescence decay after each actinic flash by an instrument described by Eaton-Rye and Govindjee (1988 a, b). An FX-124 flash lamp (EG and G, 2.5 μs duration at half-maximal peak height) was used as the saturating actinic flash. Chl *a* fluorescence yield of *Chlamydomonas* cells (10 μg Chl/mL) after a weak measuring (probe) flash (provided by the General Radio 1539 A Stroboslave flash lamp; the duration at half-maximal peak, 2.5 μs) was measured by a EMI 9558 photomultiplier tube. The measuring flash was fired at a computer-programmed time after each saturating ac-

Table 1: Effect of bicarbonate on oxygen evolution on formate-treated *Chlamydomonas* cells. Tris-acetate-phosphate medium was used as the reaction medium. The pH of the medium was 6.5. The formate (25 mM) treatment time was 3 h; concentrations of DBMIB, DMQ and ferricyanide were 0.5 μM, 1 mM and 1 mM, respectively. The concentration of re-added bicarbonate was 20 mM (pH 6.5). The standard error was calculated from 4 measurements under each condition.

Rate of oxygen evolution [μmol O_2 (mg Chla)$^{-1}$h^{-1}]				
A	B	C	C−B	(C−B)/A
$+HCO_2^-$ $-HCO_3^-$ +DMQ $+K_3Fe(CN)_6$	$+HCO_2^-$ $+HCO_3^-$ −DMQ $-K_3Fe(CN)_6$	$+HCO_2^-$ $+HCO_3^-$ +DMQ $+K_3Fe(CN)_3$		
59±0.7	100±0.7	360±7	260	4.4
		+DMBIB		
57±2	0±0	250±4	245	4.3

tinic flash; the kinetics of Chl *a* fluorescence decay was derived from these measurements. Both the actinic and measuring flashes were filtered with Corning blue CS 4-96 glass filters; and the photomultiplier was protected by the Corning red CS 2-61 filter.

Results and Discussion

A. Long-term Formate Treatment experiments

The effect of anion treatment on steady state electron transport from H_2O to DMQ

After 3 h formate treatment (25 mM formate, pH 6.5), the O_2 evolution rate of treated samples was 59 μmoles O_2/mg · Chl/h. Addition of 20 mM HCO_3^- (pH adjusted to 6.5) to this sample reversed the inhibition on PS II electron transport, enhancing the rate of oxygen evolution to 360 μmoles O_2/mg · Chl/h and, thus, showing a 6-fold enhancement in oxygen evolution by addition of bicarbonate (Table 1). This enhancement is commonly referred to as the bicarbonate effect.

The addition of 20 mM HCO_3^- to the formate-treated cells not only stimulates the rate of PS II electron flow but also increases the rate of CO_2 fixation, since there was substantial oxygen evolution (100 μmoles O_2/mg · Chl/h) without any added electron acceptor besides CO_2. The net stimulated rate, by omitting the value for carbon fixation, is 260 μmoles O_2/mg · Chl/h and the ratio between the O_2 evolution rate of HCO_2^- to HCO_2^- + HCO_3^- samples is 4.4. To exclude the influence of CO_2 fixation, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB), which blocks electron transport between photosystem II and photosystem I (Trebst et al. 1970), was used in the suspension medium. In the absence of electron acceptors and presence of 0.5 μM DBMIB no oxygen evolution was observed by bicarbonate addition. In the presence of 0.5 μM DBMIB and the electron acceptors (DMQ and ferricyanide), addition of 20 mM $NaHCO_3$ to the formate-treated cells stimulated the rate of O_2 evolution to 250 μmoles O_2/mg Chl/h. The ratio between the rate of O_2 evolution of HCO_2^- to HCO_2^- + HCO_3^- samples is 4.3, which is consistent with the above results by the substraction method.

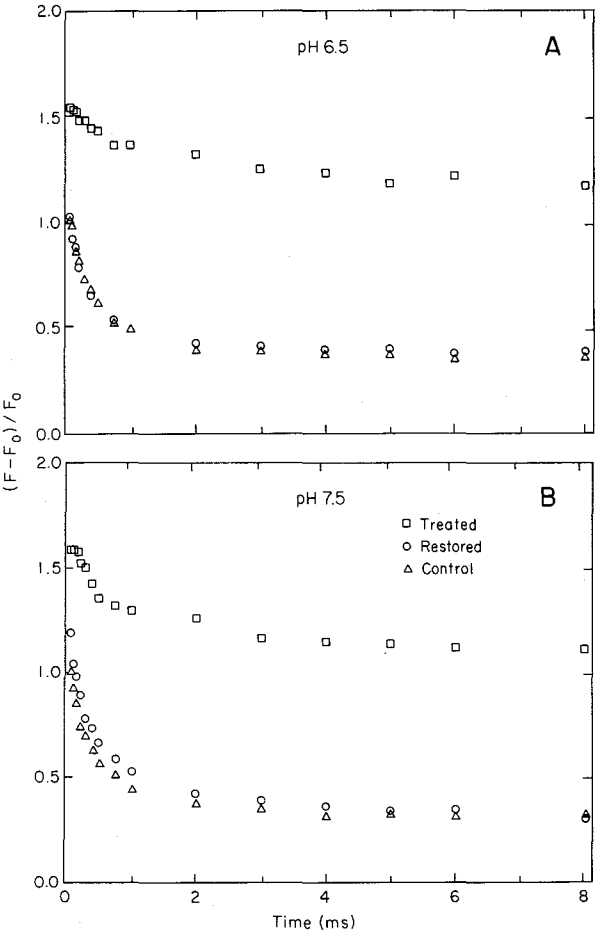

Fig. 1: The effect of long-term formate treatment on the decay of Chl *a* fluorescence yield after the third actinic flash in control (△), formate treated (□, 25 mM, 3 h) and bicarbonate-restored (○, 2.5 mM) *Chlamydomonas* cells at pH 6.5 (A) and at pH 7.5 (B). F = fluorescence yield at time t after the actinic flash; F_o = fluorescence yield before the actinic flash, i.e., when [Q_A] is maximum.

The above data clearly confirm the existence of the bicarbonate effect in *Chlamydomonas* cells: a 4-fold reversible stimulation of the rate of electron flow of photosystem II, unrelated to CO_2 fixation, was observed upon addition of bicarbonate.

Characteristics of the kinetics of Chl a fluorescence decay in Chlamydomonas cells

In order to clarify the inhibition site of long-term formate treatment, further experiments were done. Q_A, not Q_A^-, is suggested (Duysens and Sweers 1963) to be the quencher of Chl *a* fluorescence. Therefore, the decay of Chl *a* fluorescence yield is related to the increase in Q_A concentration. Chl *a* fluorescence intensity reaches maximum when Q_As are fully reduced, and approaches the lowest value when all Q_As are oxidized. The addition of herbicides, which inhibit beyond Q_A^-, moves the equilibrium in favor of the formation of Q_A^-, thus increasing the Chl *a* fluorescence yield. In

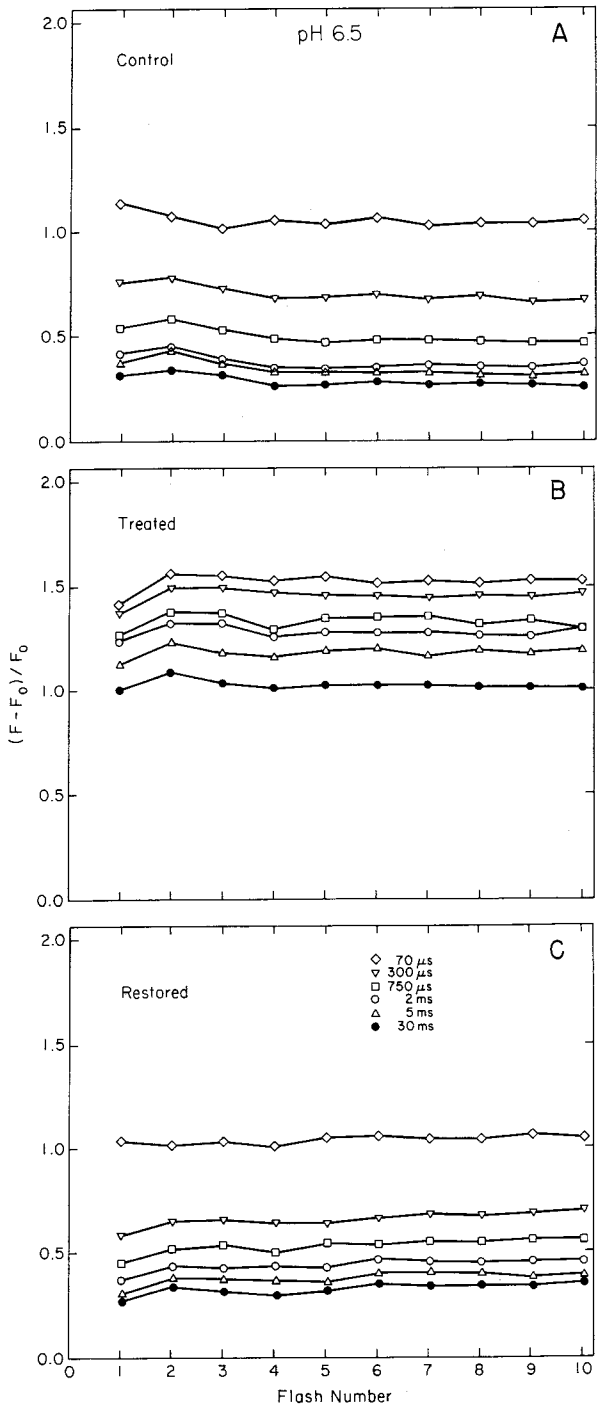

Fig. 2: Variable Chl *a* fluorescence, $(F-F_o)/F_o$, measured at different times after the flashes, as a function of flash number at pH 6.5. All the times indicated inside panel «C» are when the measuring flash was fired after the actinic flash. A, B and C represent results with control, long-term formate-treated (25 mM, 3 h) and HCO_3^- (2.5 mM) restored *Chlamydomonas* cells. For all instrumental details, see text.

the same fashion, the slowing down of Chl *a* fluorescence in the formate-treated higher plant thylakoids is interpreted as the inhibition of electron flow beyond Q_A^- (Jursinic et al. 1976). A large slowing down of Q_A^- decay occurs after three or more flashes in formate-treated higher plant thylakoids (Eaton-Rye and Govindjee 1988 a, b). Chl *a* fluorescence decays, monitoring the oxidation of Q_A^- following the third actinic flash, at pHs 6.5 and 7.5 in long-term formate-treated *Chlamydomonas* cells are presented in panels A and B, respectively (Fig. 1). At both pHs, a large slowing down of the reoxidation rate of Q_A^- due to formate treatment is observed. The addition of 2.5 mM bicarbonate (pH adjusted to 6.5 or 7.5) to the treated samples restores the kinetics to the control level. We intepret this to indicate a role for bicarbonate in the electron transport through photosystem II.

Changes in oscillation pattern of Chl *a* fluorescence intensity as a function of flash number can be used as another indicator for the existence of the bicarbonate effect in *Chlamydomonas* cells. A rather weak oscillation pattern (a binary oscillation superimposed on a four period oscillation) of the intensity of Chl *a* fluorescence (70 μs after the actinic flash) against the flash number was observed in *Chlamydomonas* cells at pH 6.5 (Fig. 2). The binary oscillation arises from the differential rates of Q_A^- oxidation by either Q_B after an odd number or Q_B^- after an even number of flashes (cf. Robinson and Crofts 1983), whereas the period four oscillation arises from the differences in the reduction rate of $P680^+$ by Z (Z being the electron donor to $P680^+$, the primary donor of PS II, see e.g., Robinson and Crofts 1983, Delsome 1971). A still weaker oscillation of Chl *a* fluorescence intensity as a function of flash number was observed in *Chlamydomonas* cells at pH 7.5 (Fig. 3). Since long-term treatment with formate blocks electron flow beyond Q_A^-, the fluorescence yields in Figs. 2 B and 3 B (treated) are much higher than in Figs. 2 A and 3 A (controls). Addition of bicarbonate restored the fluorescence intensities and oscillation pattern to those of the control (Figs. 2 C and 3 C), as it relieved the formate block on electron flow beyond Q_A.

Several components can be distinguished in the kinetics of Chl *a* fluorescence after an actinic flash. Analysis of these components can provide more information on the mechanism of formate treatment and bicarbonate effect in the green algal cells. A fast component of Chl *a* decay (half-time in the 100 μs range) and an intermediate component (half-time in the ms range) are, apparently, related to the equilibration of $Q_A^-Q_B \rightleftarrows Q_AQ_B^-$ (cf. Robinson and Crofts 1983). A slow component (half-time in the second range) may reflect the back reaction between Q_A^- and the S_2 state of the oxygen evolution complex. This may include the so-called inactive PS II centers (see Cao and Govindjee 1990). In the present study, the measurement included data up to only 8 ms after the flash. Thus, only two components (fast and intermediate) were extracted. Information on the intermediate component is qualitative, but that on the fast component is quantitative. Tables 2 and 3 show the effect of long-term formate treatment on the half-times and amplitudes of the fast (f) and intermediate (i) components of Chl *a* fluorescence decay in *Chlamydomonas* cells and *Chlamydomonas* thylakoids, respectively. The half-time of the fast component in the formate-treated cells after the third flash is about 2 times slower than that in control cells at both pHs 6.5 and 7.5 (Table 2). This was essentially reversed upon the addition

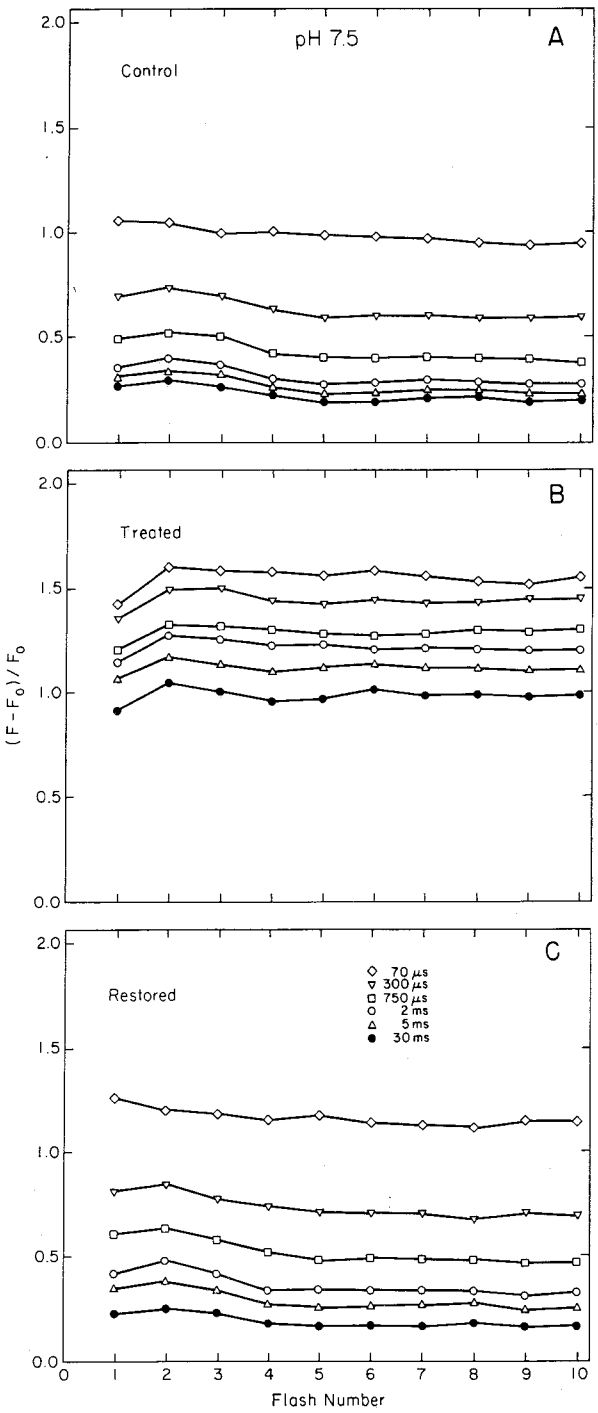

Fig. 3: Variable Chl *a* fluorescence, $(F-F_o)/F_o$, measured at different times after the flashes, as a function of flash number at pH 7.5. All the times indicated inside panel «C» are when the measuring flash was fired after the actinic flash. A, B and C represent results with control, long-term formate-treated (25 mM, 3 h) and HCO_3^- (2.5 mM) restored *Chlamydomonas* thylakoids.

of 2.5 mM HCO_3^-. Moreover, the half-time of the intermediate component is also reversibly slowed down by formate treatment. The half-time of this component in these treated cells is 1.6 and 3.3 times as much as in the control at pHs 6.5 and 7.5, respectively. Results on the half-times of the fast fluorescence decay in isolated *Chlamydomonas* chloroplasts (Table 3) are quantitatively similar to those in the cells, but no significant effect on the intermediate component was observed.

Table 2: The effect of formate treatment on the half-time ($t_{½}$) and amplitude (A) of the fast (f) or intermediate (i) components of Chl *a* fluorescence decay (after the third actinic flash) of *Chlamydomonas* cells with different treatments. For details of the instrument, see text. The time for formate treatment was 3 h; formate concentration was 25 mM. The concentration of added bicarbonate was 2.5 mM.

Treatment	pH of reaction medium	$t_{½}$ (f) (µs)	$t_{½}$ (i) (ms)	A_f (%)	A_i (%)
control	6.5	290	37	65	35
	7.5	260	24	88	32
formate-treated	6.5	560	60	20	80
	7.5	490	80	28	72
restored with bicarbonate addition	6.5	240	33	62	38
	7.5	260	20	68	32

Table 3: The effect of formate treatment on the half-time ($t_{½}$) and amplitude (A) of the fast (f) or intermediate (i) components of Chl *a* fluorescence decay (after the third actinic flash) of *Chlamydomonas* thylakoids with different treatments. For details of the instrument see text. The time for formate treatment was 3 h; formate concentration was 25 mM. The concentration of added bicarbonate was 2.5 mM.

Treatment	pH of reaction medium	$t_{½}$ (f) (µs)	$t_{½}$ (i) (ms)	A_f (%)	A_i (%)
control	6.5	440	18	75	25
	7.5	310	12	79	21
formate-treated	6.5	530	14	36	64
	7.5	480	15	49	51
restored with bicarbonate addition	6.5	360	15	78	22
	7.5	360	12	77	23

The biggest change brought about by formate treatment is on the amplitudes of both the components. Formate treatment decreased the amplitude of the fast component from about 70 % to about 25 % and increased the amplitude of the second (intermediate) component from about 30 % to about 75 % in *Chlamydomonas* cells at both pHs 6.5 and 7.5. These changes were, again, essentially reversed by the addition of 2.5 mM HCO_3^-. Isolated *Chlamydomonas* thylakoids showed results qualitatively similar to those obtained with cells. Fig. 4 shows the flash number dependence of the amplitudes of the first (fast) and second (intermediate) fluorescence decay components. Panels A and C in Fig. 4 display the amplitudes of Chl *a* fluorescence decay at pH 6.5. Panels B and D display the results at pH 7.5. Panels A and B are for *Chlamydomonas* cells and panels C and D for isolated thylakoids. All data indicate that formate treatment (triangles) induced a decrease in the amplitude of the fast component (open symbols) and an enhancement in the amplitude of the slow component (closed symbols) in both *Chlamydomonas*

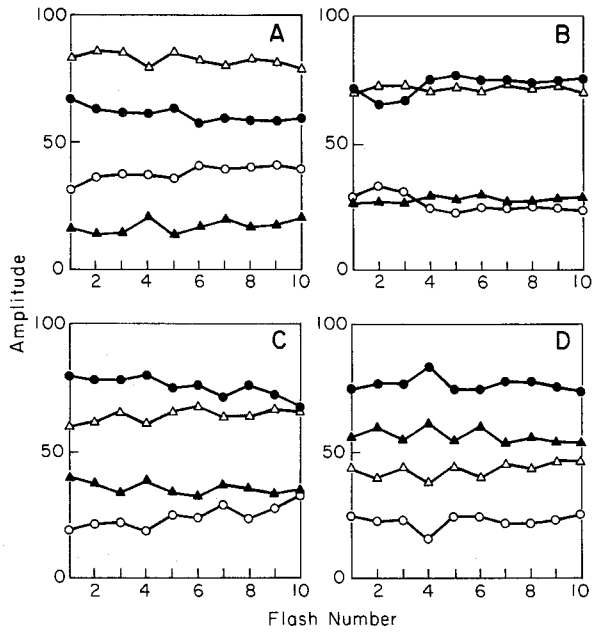

Fig. 4: Plot of the amplitudes of Chl *a* fluorescence decay at pH 6.5 (A, C) or at pH 7.5 (B, D) in *Chlamydomonas* cells (A, B) or thylakoids (C, D). ● and ○ represent data for the first (fast) and second (intermediate) components obtained from HCO_3^--restored samples, respectively. ▲ and △ represent data for the first and second components obtained from (3 h) long-term formate-treated samples, respectively.

cells and thylakoids, further confirming that a block in the electron flow between Q_A^- and Q_B was produced by formate treatment. This blockage was relieved by bicarbonate addition (circles).

B. Short-term formate treatment experiments

An action site of bicarbonate on the oxygen evolving side of the photosystem II reaction center has also been proposed (Stemler 1982). Based on measurement of the difference in Chl *a* fluorescence intensity between dark-kept and preilluminated cells and the measurement of oxygen yield, Mende and Wiessner (1985) suggested that a short-term CO_2 depletion (15 min) of green alga, *Chlamydobotrys stellata*, blocked photosynthetic electron flow at the oxidizing (electron donor) side of photosystem II. Results on chlorophyll *a* fluorescence induction after short-term (10 seconds) formate infiltration and 10 min storage of spinach leaf discs in the dark led El-Shintinawy and Govindjee (1989 b) to suggest that an inhibition of electron flow somewhere between the electron donor «Z» and Q_A was induced. In order to establish this new effect of the anion, a short-term formate treatment (5 min) was applied to *Chlamydomonas* cells in diluted tris-acetate phosphate medium. The short-term formate treatment brought about a dramatic quenching in Chl *a* fluorescence transient, implying a blockage before Q_A of photosystem II. Furthermore, addition of 10 mM bicarbonate restored Chl *a* fluorescence transient to the control level.

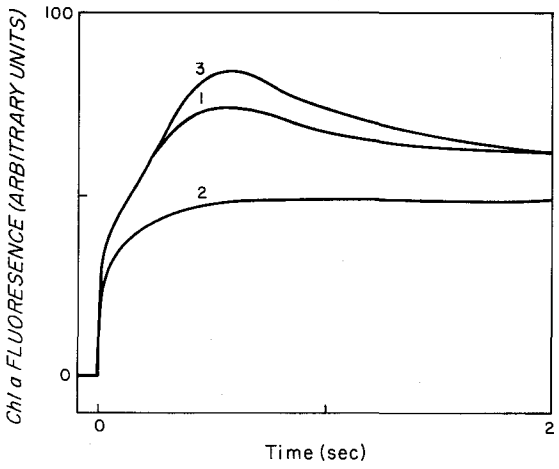

Fig. 5: Chl *a* fluorescence transient in mild-heat and hydroxylamine-treated *Chlamydomonas* cells. Trace 1: control; Trace 2: short-term formate-treated (25 mM for 5 minutes); Trace 3: restored with bicarbonate (2.5 mM). For experimental details, see Materials and Methods.

To locate the site of this inhibition, heated (45 °C, 3 min) and hydroxylamine-treated *Chlamydomonas* cells were used. Mild heating blocks O_2 evolution and hydroxylamine is an artificial electron donor to PS II. Cells were incubated for 5 min in a medium containing 25 mM formate or formate plus bicarbonate in the presence of 10 mM hydroxylamine at pH 7.3. Fig. 5 (trace 1) shows Chl *a* fluorescence transient of *Chlamydomonas* cells incubated for 5 min in diluted culture medium without addition of formate or bicarbonate. Trace 2 shows the quenching of fluorescence in formate-treated cells (5 min) that had been pretreated with heat and 10 mM hydroxylamine. Trace 3 shows the restoration of fluorescence of these cells by 10 mM HCO_3^-. This suggests that the site of inhibition by short-term formate treatment (and its reversibility by bicarbonate addition) is after the site of electron donation by hydroxylamine, i.e. between «Z» (or «D») and Q_A. We speculate that this site may be between pheophytin and Q_A.

Conclusions

In the present study, we show that bicarbonate acts to stimulate electron transport in photosystem II in the cells of green alga *Chlamydomonas* inhibited by formate treatment (Table 1). Long-term formate treatment blocked the electron flow from Q_A^- to Q_B. This blockage was observed by the slowing down of Chl *a* fluorescence decay, the dramatic decrease in amplitude of the fast decay component and the increase in amplitude of the intermediate decay component (see Fig. 1 and Tables 2 and 3) and abolition of the oscillation pattern of the fluorescence yield plotted against the flash number (Figs. 2 and 3). Addition of bicarbonate to the formate-treated samples restores chlorophyll *a* fluorescence decay, the amplitudes of different components and the oscillation pattern of the fluorescence to the control level. At

Table 4: The effect of formate treatment on the ratios of half-time (Rt) and amplitude (RA) of fast (f) or intermediate (i) components of Chl *a* fluorescence decay (after the third actinic flash) of *Chlamydomonas* cells and thylakoids. All data were calculated from Tables 2 and 3. All experimental conditions were the same as specified there.

pH	Ratio (treated/restored)	cells	chloroplasts
6.5	Rt_f	2.4	1.5
	Rt_i	1.8	0.9
	RA_f	0.3	0.5
	RA_i	2.1	2.9
7.5	Rt_f	1.9	1.3
	Rt_i	4.0	1.3
	RA_f	0.4	0.6
	RA_i	2.3	1.8

pH 6.5, the amplitude of the fast component (A_f) of HCO_3^--restored cells is 2–3 times larger than that of formate-treated thylakoids or cells. In contrast, the amplitude of the intermediate component of HCO_3^--restored cells and thylakoids is 2–3 times smaller than that of formate treated samples. These and similar data at pH 7.5 (Table 4) show that long-term formate treatment of *Chlamydomonas* thylakoids and cells causes a remarkable bicarbonate-reversible inhibition of the electron flow between Q_A^- and Q_B on the electron acceptor side of PS II.

Mende and Wiessner (1985) discovered that CO_2-depletion caused different effects depending upon the time of CO_2 depletion in a green alga, *Chlamydobotrys stellata*. After prolonged depletion (>15 min), CO_2 depletion mainly blocked the electron flow beyond Q_A; however, in a short-term range (<15 min) of depletion, a blockage on the oxidizing side of PS II was suggested. They did not see this effect in *Chlorella*. El-Shintinawy and Govindjee (1989 b) succeeded in reproducing this observation in spinach discs under different experimental conditions. The latter authors used the formate infiltration method for CO_2-depletion; under this condition, only 10 seconds was needed for the short-term treatment. In this paper, the existence of a site for formate treatment before Q_A is suggested also for *Chlamydomonas* cells. Using spinach thylakoids, Xu, Taoka, Crofts and Govindjee (unpublished, 1989) have found this effect of formate binding to be pH dependent.

It is not yet certain whether formate binds to empty sites as suggested by Stemler (1989), to sites where HCO_3^- is bound *in vivo* as suggested by Blubaugh and Govindjee (1988 a) or has different exchange mechanisms at different sites. It is, however, logical to believe that at the alkaline pH of the stroma, HCO_3^- will be bound at all of the sites *in vivo*. In view of differences observed between results at pHs 6.5 and 7.5 (see Tables 2 and 3), it is suggested that these effects may be related to the protonation of Q_B^-, as suggested earlier by Eaton-Rye and Govindjee (1988 a, b) and Blubaugh and Govindjee (1988 a).

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