Photosynthesis Research **27**: 199–208, 1991.

Regular paper

The herbicide-resistant D1 mutant L275F of *Chlamydomonas reinhardtii* fails to show the bicarbonate-reversible formate effect on chlorophyll *a* fluorescence transients

Govindjee[1], Beatrix Schwarz[2], Jean-David Rochaix[3] & Reto J. Strasser[2]
[1]*Departments of Physiology & Biophysics and Plant Biology, University of Illinois, 505 South Goodwin Avenue, 289 Morrill Hall, Urbana, IL 61801-3793, USA;* [2]*Bioenergetics Laboratory, University of Geneva, CH 1254 – Lullier-Jussy, Switzerland;* [3]*Departments of Molecular Biology and Plant Biology, University of Geneva, CH 1211 Geneva, Switzerland*

Received 5 July 1990; accepted in revised form 21 December 1990

Key words: Photosystem II, herbicides, mutants, D-1 protein, bicarbonate, (*Chlamydomonas reinhardtii*)

Abstract

Herbicide-resistant mutants of the eukaryotic green alga *Chlamydomonas reinhardtii*, that are altered in specific amino acids in their D-1 protein, show differential bicarbonate-reversible formate effects. These results suggest the involvement of D1 protein in the 'bicarbonate effect'. A 25 mM formate treatment of mixotrophically or photoautotrophically grown wild type cells results in a slower rise of chlorophyll *a* fluorescence transient followed by a dramatically slowed decline during measurements in continuous light. These effects are fully reversed upon addition of 10 mM bicarbonate. The mutant BR-202 [L275F] is, however, highly insensitive to 25 mM formate suggesting that a significant change in formate (bicarbonate) binding has occurred in helix V of the D1 protein near histidine involved in Fe binding. With the exception of DCMU-4 [S264A], which is considerably more sensitive to formate than the wild type, five other different [V219I, A25IV, F255Y, G256D and cell-wall deficient CW-15] mutants display a relatively similar response to formate as wild type. Absence of formate effect on a PS II-lacking [FuD-7] mutant confirms the sole involvement of PS II in the 'bicarbonate effect'.

Introduction

Photosystem II (PS II) acts as a water-plastoquinone oxido-reductase; using four photons it transfers four electrons from 2 molecules of water to plastoquinone producing molecular O_2 and two molecules of doubly reduced plastoquinone (see a review by Hansson and Wydrzynski 1990). During this process, water protons are released into the lumen. Additional protons are taken up into the thylakoid membrane from its stromal side and utilized to produce plastoquinol from the doubly reduced plastoquinone (see reviews by Vermaas and Govindjee 1981, and Crofts and Wraight 1983, and Govindjee and Wasielewski 1990).

It has been suggested that one of the functions of bicarbonate is to regulate the reduction of plastoquinone beyond Q_A, the one-electron primary plastoquinone acceptor of PS II, observed most clearly after the third and subsequent flashes in isolated thylakoids from higher plants (see e.g., Govindjee et al. 1976, Robinson et al. 1984, van Rensen et al. 1988, Eaton-Rye and Govindjee 1988a,b). In addition, a bicarbonate-reversible formate effect has been observed in spinach leaf discs and in *Chlamydomonas reinhardtii* that is suggested to occur prior to Q_A

(El-Shintinawy and Govindjee 1990, El-Shintinawy et al. 1990, cf. Govindjee et al. 1989). These bicarbonate effects in oxygenic photosynthesizers including cyanobacteria (Cao and Govindjee 1988) are assumed to occur through the binding of HCO_3^- to the reaction center II complex (Blubaugh and Govindjee 1988). The Fe between Q_A and Q_B has been shown to play an important role in this bicarbonate-reversible formate effect (Diner and Petrouleas 1990). Such effects, however, do not exist in purple photosynthetic bacteria (Shopes et al. 1989) and in green bacterium *Chloroflexus aurantiacus* (Govindjee, J. Trost and R. Blankenship, unpublished, 1990). This may be due to differences in the architecture of the reaction center proteins D1 and D2 of plants and cyanobacteria and of L and M subunits of photosynthetic bacteria (Michel and Deisenhofer 1988). Recently, we have shown (Govindjee et al. 1990) that different D1 mutants of the prokaryotic cyanobacteria *Synechocytis* 6714 are differentially sensitive to bicarbonate-reversible formate effects, suggesting a possible interaction of HCO_3^- with the D-1 protein. The availability of similar, yet different, herbicide resistant D1 mutants of the eukaryotic alga *C. reinhardtii* (see Erickson et al. 1989) has allowed us to test the idea of these possible interactions of bicarbonate with the D-1 protein in an eukaryotic system. *C. reinhardtii* has been extensively used as a tool for studying photosynthesis (Whitmarsh and Togasaki 1986). Since it is amenable both to genetic and biochemical analysis (Harris 1988), it is a choice organism to study the bicarbonate-reversible formate effect.

In this paper, we have studied the bicarbonate-reversible formate effect on several herbicide-resistant D1 mutants of *C. reinhardtii* cells in order to understand the relationship of the herbicide-binding niche with possible formate and, thus, of bicarbonate binding sites. Since formate slows down the chlorophyll *a* fluorescence rise from the characteristic point D to P in the fluorescence transient (suggestive of a block prior to the plastoquinone pool; low Chl *a* fluorescence is related to the concentration of Q_A, not Q_A^-, Duysens and Sweers 1963), followed by a high yield of fluorescence in the P to S decline region (suggestive of a block beyond Q_A), we have used fluorescence transients to characterize the bicarbonate-reversible formate effect in this paper.

Materials and methods

Cultivation of algae. Chlamydomonas reinhardtii wild type, several herbicide-resistant mutant strains (see Table 1) and two others (a cell-wall deficient mutant CW-15 and a PS II-lacking mutant FuD-7) were used in this study. These strains were grown either mixotrophically in Tris-acetate phosphate (TAP) or photoautotrophically in Tris-phosphate (TP) medium (Gorman and Levine 1965). Mixotrophic cultures were grown for 24 h in continuous light (Lumiere Silvana coolwhite 4300 F30W/TB/CW; light intensity, 5 W m^{-2}). Photoautotrophic cultures were grown for 2–4 days in a 12:12 h light/dark cycle at 20°C in a culture chamber fitted with Phillips lamp (TLD 36W/33; light intensity, 5 W m^{-2}).

Table 1. Description of D-1 mutants used in this paper

Mutant*	Name of the mutant	Resistant to herbicides		
		Atrazine	Bromacil	Diuron
V219I	Dr-2, Dr-18	2 X	2 X	20 X
A251V	MZ-2	25 X	1000**	5 X
F255Y	AR-207	15 X	1 X	0.5 X
G256D	AR-204	15 X	10 X	3 X
S264A	DCMU-4	100 X	250 X	20 X
L275F	BR-202	1 X	5 X	5 X

* Mutants are listed with the original amino acid followed by codon number and the mutated amino acid. Data on all mutants, except MZ-2, are from Erickson et al. (1989); data for MZ-2 is from Johanningmeir et al. (1987).
** Data for metribuzin, not bromacil.

Fluorescence transient measurements. Chlorophyll *a* fluorescence transients were measured by a home-built spectroflurometer equipped with a three-armed fiber-optics light guide (Strasser and Sironval 1972). Chlorophyll *a* fluorescence was excited by 50 W m^{-2} 632.8 nm light from a He–Ne laser. Front surface fluorescence, that passed through a combination of a red cut-off Corning CS 2-64 glass and a Balzer B40 interference filter transmitting 690 nm (full width at half height, 10 nm; 35% transmission at the peak), was monitored by a red-extended S-20 Hamamatsu R928 photomultiplier tube. The time course of fluorescence was displayed on a 10 MHz storage oscilloscope (Tektronix T912, Tektronix Guernsey Ltd, C.I.) When needed, fluorescence transients were plotted on a X–Y recorder (Servogor XY-733 recorder, BBC Goerz). A basic earlier version of this spectrofluorometer is described by Karapetyan et al. (1983).

A 300 μl sample was placed between two discs of 0.7 mm thick plexiglas (diameter, 17 mm) separated by a 2 mm teflon spacer (diameter, 14 mm) that constituted part of a vertical cuvette.

Chlorophyll concentration of the sample was adjusted to 1 to 2 μg/ml suspension. Samples were dark-adapted, as described later, before fluorescence transients were monitored. The shutter of the instrument was fully open at 4 ms; fluorescence intensity at 4 ms is F initial, and is estimated to be close to the F_o level within 5% (i.e., fluorescence intensity when all Q_A is in the oxidized state).

Measurements of fluorescence in modulated light. Chl *a* fluorescence emission (at 685 nm) from *C. reinhardtii* cells was measured at room temperature using a Hansatech modulated twin channel instrument attached to a micro-processor power supply (Electro-Automatik, model EA-3040), as described by Havaux et al. (1991). Excitation was with a modulated yellow light (peak wavelength, 585 nm; modulation frequency, 870 Hz) provided by an array of yellow light-emitting diodes combined with an Ealing Beck 35-5404 filter. Fluorescence, that passed through a 685 nm interference filter, was detected with a photodiode. The intensity of the modulated light was low enough as not to induce any significant variable fluorescence, so that the fluorescence level recorded was close to the fluorescence level F_o. Short (1 s) pulses of intense blue light (500 W m^{-2}) were used to measure the maximal level F_m of modulated Chl fluorescence. Chl *a* fluorescence transient was measured in a non-modulated (actinic) beam of blue light, the intensity of which was adjusted to 45 W m^{-2}. This non-modulated light was supplied by a Schott KL1500 light source combined with a 320 to 620-nm broad-band blue filter; it was delivered, via fiber optics, to the bottom of the vial containing the cells, fitted to the Hansatech 'leaf-clip'. The fluorescence signals were displayed on a potentiometric chart recorder or stored on diskettes.

All light intensities were measured with a YSI-Kettering 65A radiometer.

Cells and their treatment. We recognized at the beginning of our study that it was important to keep *C. reinhardtii* cells shaking prior to measurements. If they were kept in stationary conditions, chlorophyll *a* fluorescence transients were lost. We also recognized that addition of 25 mM formate to *C. reinhardtii* cells in their mixotrophic growth medium (Tris-acetate phosphate, TAP, buffer) at pH 7.0 or 7.5 gave no effects in any of the cells examined. This is due to the pH optima of the effect being at pH 6.5, and a possible competition between formate and acetate (cf. Blubaugh and Govindjee 1988). Furthermore, addition of 100 mM formate to cells usually inhibited chlorophyll *a* fluorescence that could only be partially recovered by bicarbonate addition. Thus, in this study, we treated *C. reinhardtii* cells, suspended in half-diluted tris-phosphate (TP) medium, with 25 mM formate at pH 6.5, unless otherwise stated. This treatment gave the most reproducible bicarbonate-reversible formate effect.

Chlorophyll determination. A standard curve relating chlorophyll concentration, obtained by 80% acetone extract of cells, to the absorbance at 680 nm was obtained for *C. reinhardtii* cells. This curve was perfectly linear up to 12 μg Chl/ml, used in this study. Afterwards, absorbance at 680 nm (A680) provided information on

[Chl] of samples; in all measurements Perkin–Elmer Lambda 3 UV/VIS Spectrophotometer was set at zero absorbance at 800 nm. The empirical relationship for wild type cells was: μg total chlorophyll/ml suspension = 29.43 × A680.

Results and discussion

Wild type cells. Figure 1 shows the chlorophyll *a* fluorescence transients of wild type (WTd^-) cells of *C. reinhardtii* that were grown photoautotrophically in TAP medium at pH 7.0 and resuspended in half-diluted TP medium at pH 6.5. The normal OIDPS fluorescence transient (Govindjee and Papageorgiou 1971, Spalding et al. 1984, Govindjee and Statoh 1986) is observed in the control cells. After 6 min of dark incubation with 25 mM formate, the P to S decay is replaced by the fluorescence locked at the 'P' level (not shown). After 4 min of further incubation, the I to P rise is also slowed (Fig. 1). Both of these effects are restored upon the addition of 10 mM bicarbonate. These results indicate that either the reduction of Q_A is slowed and/or the reduction of the plastoquinone pool occurs more slowly upon formate treatment. Although the delayed decay of P to S is usually more difficult to interpret because it may be related to several phenomena (Briantais et al. 1986, Govindjee and Satoh 1986), it is, in our sample, due to inhibited (or slowed) Q_A^- to Q_A reaction based on fluorescence decay measurements (El-Shintinawy et al. 1990) and on fluorescence measurements in modulated light (see below).

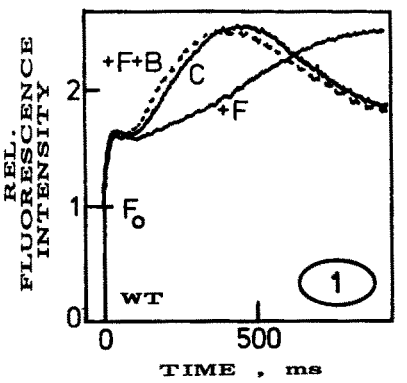

Fig. 1. Chlorophyll *a* fluorescence transient of wild type *C. reinhardtii* cells suspended in tris-phosphate medium at pH 6.5. C for photoautotrophically grown cells (see text for details), +F for 25 mM formate treated cells, and +F + B for 25 mM formate followed by 10 mM bicarbonate treated cells. Dark adaptation, 4 min but see text for further details. The ratio of variable fluorescence level (F_s–F_o) at 1 s after illumination with and without formate was 1.4 ± 0.05 (n = 3).

In order to test if the formate-induced absence (or decrease) of the fluorescence decay in the P to S phase is due to the accumulation of Q_A^-, or due to other causes (such as non-photochemical effects), we measured fluorescence yield by a weak modulated light (for details, see *Materials and methods*, and the legend of Fig. 2). The weak modulated light alone measures a level close to F_o that is independent of photochemistry as almost all Q_A remains in the oxidized state here. However, this weak modulating light measures F_m during excitation of the actinic pulse (FL in Fig. 2). Results with control *C. reinhardtii* cell suspension show (Fig. 2, top left) that the ratio of F_m/F_o is 2.7, and that even during P to S decay during 12 s of non-modulated illumination, almost the same F_m is reached when the actinic pulse is given. Thus, the P to S decay in *C. reinhardtii* cells during the 10 s time range is mostly photochemical and due to conversion of Q_A^- to Q_A. Formate treatment (Fig. 2, top right) causes a reduction in F_m/F_o, as expected due to a partial blockage prior to Q_A (see El-Shintinawy et al. 1990). Also, the fluorescence rise during exposure to non-modulated beam is slower. However, fluorescence remains high and the exposure to actinic light causes only a slight increase. The fluorescence level, F_m, measured during the P to S phase is the same as before the transient measurement. This shows the existence of the second effect, the block after $[Q_A^-]$; it remains high as formate treatment causes accumulation of $[Q_A^-]$. After the non-modulated light was turned off, the fluorescence decay was extremely slow. This conclusion was confirmed by observing that addition of 0.3 μM diuron, that is known to block electron flow beyond Q_A^-, shows similar results (Fig. 2, bottom right). However, the fluorescence rise in non-modulated light is faster in the diuron case than in the formate case since there is no blockage prior to Q_A in the case of diuron. It is important to point out that the measured F_o level in the diuron

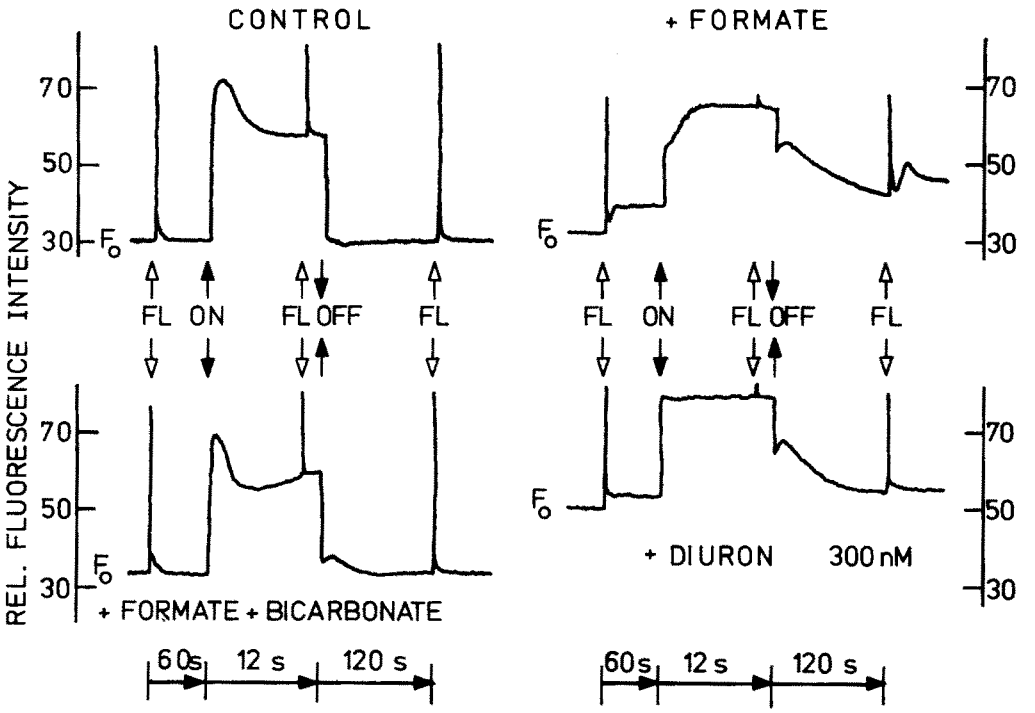

Fig. 2. Modulated fluorescence experiments with wild type *C. reinhardtii* cells. Data on control cells are shown on the top left corner; + Formate samples (top right) are those to which 25 mM formate was added; + formate + bicarbonate (bottom left) are those to which 10 mM bicarbonate was added after the formate treatment; and +Diuron samples are those to which 0.3 μM DCMU was added with or without formate. Each experiment had the same following protocol: First, F_o was recorded with a weak 585 nm modulated (870 Hz) measuring beam only; this light remained 'on' throughout the experiment; at 'FL', a pulse of 1 s 500 W m^{-2} blue light was given to measure F_m; after 1 min darkness at 'ON', a 45 W m^{-2} non-modulated blue light was given for 12 s; during the last seconds of this light, F_m was again measured; the non-modulated light was turned off for 2 min before F_m was measured for the last time.

sample is definitely distorted* due to the faster accumulation of Q_A^- even in the weak light used in this experiment. Addition of 10 mM bicarbonate (Fig. 2, bottom left) to the formate treated sample restores the sample to the control case. Addition of diuron to this sample gave the same curve as that obtained when diuron is added to control cells. The above experiments establish that a dual bicarbonate-reversible formate effect exists in *C. reinhardtii* cells, one before and one after Q_A.

L275F mutant. Since it has been suggested that bicarbonate may bind to Fe (Michel and Deisenhofer 1988, Blubaugh and Govindjee 1988, Diner and Petroulaes 1990), we chose a mutant affected in helix V of D1 (Trebst 1987) in which leucine 275, that is quite close to histidine suggested to bind Fe, is changed to phenylalanine. This mutant, Br-202, is 5 times more resistant to bromacil and diuron than the wild type (Erickson et al. 1989; also see Table 1), and it did not display the bicarbonate-reversible formate effect when treated with 25 mM formate (Fig. 3). The data suggest that the environment of the Fe-binding region has a strong influence on formate binding. It appears that in this mutant either formate (and/or HCO_3^-) is unable to bind or bicarbonate is very tightly bound. In either case, this result is supportive of the hypothesis that a binding site near Fe on the D-1 protein may play a significant role in the regulation of electron flow from Q_A to Q_B by bicarbonate.

* The measured F_o is above the true F_o and points out the difficulty of measuring true F_o when the rate of reduction of Q_A is fast as is also the case in inactive PS II centers (cf. Cao and Govindjee 1990); under such conditions, even the use of far red light to measure F_o is not an adequate procedure!

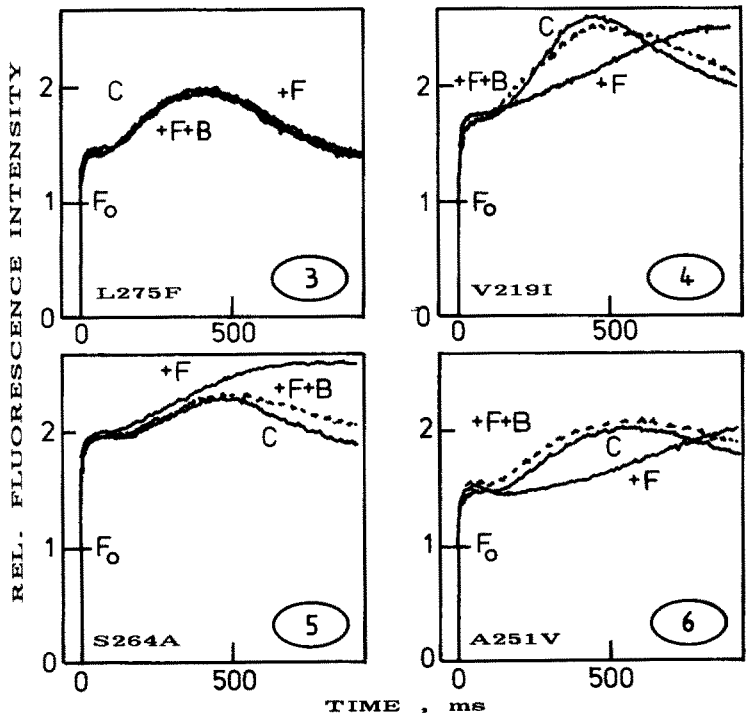

Fig. 3. Chlorophyll *a* fluorescence transient of L275F [Br-202] *C. reinhardtii* cells. The ratio of F_s–F_o at 1 s after illumination with and without 25 mM formate was 1.00 ± 0.02 ($n = 3$).

Fig. 4. Chlorophyll *a* fluorescence transient of V219I [Dr-18] *C. reinhardtii* cells. The ratio of F_s–F_o at 1 s after illumination with and without 25 mM formate was 1.48 ± 0.05 ($n = 3$).

Fig. 5. Chlorophyll *a* fluorescence transient of S264A [DCMU-4] *C. reinhardtii* cells. The ratio of F_s–F_o at 1 s after illumination with and without 25 mM formate was 2.00 ± 0.02 ($n = 3$).

Fig. 6. Chlorophyll *a* fluorescence transient of A251V [MZ-2] *C. reinhardtii* cells. The ratio of F_s–F_o at 1 s after illumination with and without 25 mM formate was 1.10 ± 0.05 ($n = 3$).

V219I mutant. Two of these mutants, Dr-2 and Dr-18 carry this mutation which changes valine 219 to isoleucine (Erickson et al. 1989). These mutants are 2 X as resistant to atrazine, 0.4 X as resistant to bromacil and 4 X as resistant to diuron than L275F [Br-202]. The changed amino acid is in helix IV, located close to the other histidine (215) suggested to bind to Fe (Trebst 1987). In contrast to the L275F mutant, the V219I mutants (both Dr-2 and Dr-18) were found to be almost as or more sensitive than wild type (Fig. 4 for Dr-18). Fluorescence transients for Dr-2, obtained at three different pHs (pH 5.8, 6.5 and 7.0), confirmed that the optimum bicarbonate-reversible formate effect was indeed at pH 6.5 (data not shown).

S264A mutant. This mutant, DCMU-4, is the most resistant of all herbicide resistant mutants examined (100 X to atrazine, 250 X to bromacil and 20 X to diuron). The changed amino acid is in the loop between helix IV and V. Its chlorophyll *a* fluorescence transient is quite different from all the others used (Fig. 5). The ratio of OP/OI level is lowest (1.3 to 1.6), whereas the others showed a ratio of 2.0 to 3.0. In spite of these differences, this mutant was very sensitive to formate. It is more sensitive than the wild type for the effect beyond Q_A. The ratio of fluorescence level at 1 s after illumination with formate to that without formate was highest in DCMU-4 than in any other mutant or wild type.

A251V mutant. To test if the high sensitivity of S264A [DCMU-4] is simply due to the location of the altered amino acid in the exposed loop between helix IV and V, or is a more specific phenomenon, we chose the mutant A251V [MZ-

2] (Johanningmeir et al. 1987) where alanine at position 251 is changed to valine in a helix parallel to the membrane. The 25 mM formate effect was much less pronounced here than for the S264A mutant and even for the wild type (Fig. 6). Chlorophyll *a* fluorescence continued to rise beyond the P level of the control suggesting a block beyond Q_A; the effect prior to Q_A was also visible in A251V [MZ-2] mutant.

F255Y mutant. In continuation of our tests for the role of amino acids in the loop between helix IV and V, we used the mutant F255Y [Ar-207] that was much more sensitive to diuron than the A251V [MZ-2] mutant. This mutant seems to be more sensitive to 25 mM formate than A251V, but as sensitive as V219I [Dr-18]: DP rise was slowed and the S level was high (Fig. 7).

G256D mutant. Changes at amino acid 256 in mutants Ar-204 and Br-24 are right next to F255Y [Ar-207] mutant; G256D mutant is much more resistant to bromacil than F255Y, but is equally resistant to Atrazine (cf. Table 1). Although it has a lower variable fluorescence than F255Y, it has similar sensitivity to formate. Thus, results on F255Y and G256D (both mutations in the loop between helix IV and V) show no significant difference with the wild type with regard to formate sensitivity. This suggests absence of formate/bicarbonate binding site in this region. However, binding of HCO_3^- to arginine 269, that is close to leucine 275 (low formate effect in L275F mutant) and serine 264 (large formate effect in S264A mutant), is a possibility (discussions in Blubaugh and Govindjee 1988). We suggest that such a possibility should be tested through site-directed mutagenesis of this R-269.

Mutant lacking PS II or PS I. Using FuD-7 mutant, that lacks the PS II complex (Bennoun et al. 1986), we have tested if any of the formate effects observed in this paper is unrelated to active PS II reaction center complex. As expected, this mutant had no variable fluorescence, and, thus, showed no effect of formate (Fig. 8). Using the FuD-3 mutant, that lacks the PS I reaction center complex, we observed a pronounced variable fluorescence from the O to the P level where it reached a plateau. Addition of 25 mM formate slowed this rise kinetics, and further addition of 10 mM bicarbonate reversed this effect (data not shown). This clearly establishes that both bicarbonate-reversible formate effects are located in PS II.

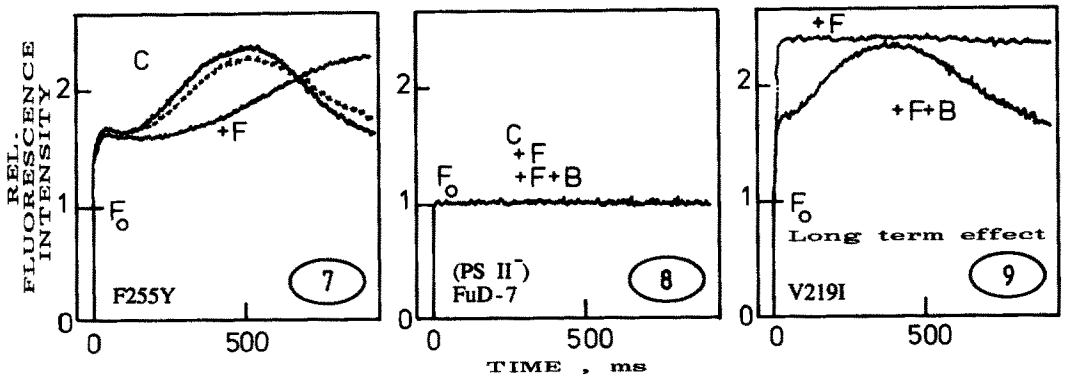

Fig. 7. Chlorophyll *a* fluorescence transient of F255Y [Ar-207] *C. reinhardtii* cells. The ratio of F_s–F_o at 1 s after illumination with and without 25 mM formate was 1.47 ± 0.05 ($n = 3$).

Fig. 8. Chlorophyll *a* fluorescence transient of photosystem II – lacking *C. reinhardtii* (FuD-7) cells. See text for details. There was no variable fluorescence and no effect of formate.

Fig. 9. Chlorophyll *a* fluorescence transient of V219I [Dr-18] *C. reinhardtii* cells with and without long-term formate treatment (see text for details). The initial F level was raised upon formate treatment as in the case of DCMU treatment, and the fluorescence reached and stayed at the P level suggesting a block in electron flow beyond Q_A. Wild type cells gave almost identical results.

Cell-wall deficient mutant (CW-15). In order to test if there was any barrier to formate due to the cell walls, we used the cell-wall deficient mutant CW-15 (Davies and Plaskitt 1971). No significant difference was observed between the results on CW-15 (data not shown) and the wild type suggesting the absence of barrier for the entry of formate into the cells.

Long-term formate treatment. Although we have not studied long-term formate treatment in this paper, it was necessary to check if our samples showed the well known effect of formate beyond Q_A (Blubaugh and Govindjee 1988). This occurred (see Fig. 9) for data from V219I [Dr-18] cells that were treated for a couple of hours with 30 mM formate under CO_2-free N_2 gas. Here, formate-induced increases in the initial F levels, as is often the case when the Q_B^-/Q_B ratio is high in cells in dark and under anaerobiosis (Govindjee et al. 1990).

Concluding remarks

On the basis of variable chlorophyll *a* fluorescence level at 1 s after illumination ($F_{1s} - F_o$), sensitivity to formate followed the order (highest to lowest): S264A [DCMU-4] ≫ V219I [Dr-2, Dr-18] ~ F255Y (Ar-207) ~ G256D (Ar-204) ~ wild type ≫ A251V [MZ-2] > L275F [Br-202] in *C. reinhardtii* cells. A 25 mM formate treatment failed to elicit any effect in L275F mutant suggesting the importance of an amino acid near Fe-liganding histidine in helix V in the formate/bicarbonate effect. These results clearly establish the importance of D1 in bicarbonate-reversible formate effect in PS II.

It is important to point out that, at air levels, there is plenty of HCO_3^- at pH of the stroma to remain bound in the native membrane (Blubaugh and Govindjee 1988). Furthermore, Govindjee et al. (1991) have established that, at pH 6.5, formate does indeed release CO_2 from thylakoid membranes and cause inhibition of electron flow in the minute range. We expect the same to be true for *C. reinhardtii* cells. Further research is necessary to unravel the molecular mechanism of the bicarbonate effects in *C. reinhardtii* cells.

Our current hypothesis for the bicarbonate effect is that bicarbonate binds to more than one entity (Fe/specific arginine/lysine) at the reaction center II proteins. The binding involves both D1 (Govindjee et al. 1991, this paper) and D2 (J. Cao, W. Vermaas and Govindjee, unpublished) proteins and affects the stability of the PS II reaction center and, thus, electron flow and protonation on the electron acceptor side in a complicated manner (Blubaugh and Govindjee 1988), the nature of which still needs to be discovered. Nitric oxide (Diner and Petrouleas 1990) and formate access different but overlapping bicarbonate binding niches. Site-directed mutagenesis of both D1 and D2 proteins is expected to aid in our understanding of the molecular basis of this 'bicarbonate effect' that may include protection against photoinactivation (see e.g., Sundby et al. 1989).

Acknowledgements

Govindjee thanks the University of Illinois at Urbana for Associate Membership in the Center for Advanced Studies and the Interdisciplinary McKnight grant during the period of this research. He is also grateful to the Academy of Science of Geneva and the University of Geneva for financial support through the office of Reto J. Strasser. Part of this work was supported by grants of the Swiss National Science Foundation (Nr 31-26345.89 to J.D. Rochaix and Nr 31-27799.89 to R.J. Strasser). We thank Jeannette von Dillerwijn for supplying us with all the mutants and Jeanne Kroese for the chlorophyll calibration of cell extracts and in vivo absorption measurements of *C. reinhardtii.*

References

Bennoun P, Spierer-Herz M, Erickson J, Girard-Bascou J, Pierre Y, Delosme M and Rochaix J-D (1986) Characterization of photosystem II mutants and *Chlamydomonas reinhardtii* lacking the psbA gene. Plant Mol Biol 6: 151–160

Blubaugh D and Govindjee (1988) The molecular mechanism of the bicarbonate effect at the plastoquinone reductase site of photosynthesis. Photosynth Res 19: 85–128

Briantais J-M, Vernotte C, Krause GH and Weis E (1986)

Chlorophyll *a* fluorescence of higher plants: Chloroplasts and leaves. In: Govindjee, Amesz J and Fork DC (eds) Light Emission by Plants and Bacteria, pp 539–583. Academic Press, Orlando, FL

Cao J and Govindjee (1988) Bicarbonate effect on electron flow in a cyanobacterium *Synechocystis* PCC 6803. Photosynth Res 19: 277–285

Cao J and Govindjee (1990) Chlorophyll *a* fluoroescence transient as an indicator of active and inactive photosystem II in thylakoid membranes. Biochim Biophys Acta 1015: 180–188

Crofts AR and Wraight C (1983) The electrochemical domain of photosynthesis. Biochim Biophys Acta 726: 149–185

Davies DR and Plaskitt A (1971) Genetical and structural analyses of cell wall formation in *Chlamydomonas reinhardtii*. Genet Res 17: 33–43

Diner BA and Petrouleas V (1990) Formation by NO of nitrosyl adducts of redox components of the Photosystem II reaction center II. Evidence that HCO_3^- /CO_2 binds to the acceptor-side non-heme iron. Biochim Biophys Acta 1015: 141–149

Duysens LNM and Sweers HE (1963) Mechanism of two photochemical reactions in algae as studied by means of fluorescence. In: Jap Soc of Plant Physiol (eds) Studies on Microalgae and Photosynthetic Bacteria, pp 353–372. Tokyo: The University of Tokyo Press

Eaton-Rye JJ and Govindjee (1988a) Electron transfer through the quinone acceptor complex of photosystem II in bicarbonate-depleted spinach thylakoid membranes as a function of actinic flash number and frequency. Biochim Biophys Acta 935: 237–247

Eaton-Rye JJ and Govindjee (1988b) Electron transfer through the quinone acceptor complex of photosystem II after one or two actinic flashes in bicarbonate-depleted spinach thylakoid membranes. Biochim Biophys Acta 935: 248–257

El-Shintinawy F and Govindjee (1990) Bicarbonate effects in leaf discs from spinach. Photosynth Res 24: 189–200

El-Shintinawy F, Xu C and Govindjee (1990) A dual bicarbonate reversible formate effect in *Chlamydomonas* cells. J Plant Physiol 136: 421–428

Erickson JM, Pfister K, Rahire M, Togasaki RK, Mets L and Rochaix J-D (1989) Molecular and biophysical analysis of herbicide-resistant mutants of *Chlamydomonas reinhardtii*: Structure-function relationship of the photosystem II D1 polypeptide. The Plant Cell 1: 361–371

Gorman DS and Levine RP (1965) Cytochrome f and plastocyanin: Their sequence in the photosynthetic electron transport chain of *Chlamydomonas reinhardtii*. Proc Natl Acad Sci USA 54: 1665–1669

Govindjee and Papageorgiou G (1971) Chlorophyll fluorescence and photosynthesis: Fluorescence transient. In: Giese AC (ed) Photophysiology, Vol 6, pp 1–50. Academic Press, New York

Govindjee and Satoh K (1986) Fluorescence properties of chlorophyll *b*- and chlorophyll *c*-containing algae. In: Govindjee, Amesz J and Fork DC (eds) Light Emission by Plants and Bacteria, pp 497–537. Academic Press, Orlando, FL

Govindjee and Wasielewski MR (1989) Photosystem II from a femtosecond to a millisecond. In: Briggs WR (ed) Photosynthesis, pp 71–103. Alan R. Liss Inc., N.Y.

Govindjee, Pulles MPJ, Govindjee R, van Gorkom HJ and Duysens LNM (1976) Inhibition of the reoxidation of the secondary acceptor of photosystem II by bicarbonate depletion. Biochim Biophys Acta 449: 602–605

Govindjee, Robinson H, Crofts AR and van Rensen JJS (1989) Bicarbonate does not influence electron transport to the reaction center chlorophyll *a* of photosystem II: Measurements by chlorophyll *a* fluorescence rise in microsecond. Naturwissenschaften 76: 719–721

Govindjee, Vernotte C, Peteri B, Astier C and Etienne A-L (1990) Differential sensitivity of bicarbonate-reversible formate effects on herbicide-resistant mutants of *Synechocystis* 6714. FEBS Lett 267: 273–276

Govindjee, Weger HG, Turpin DH, van Rensen JJS, de Vos OJ and Snel JFH (1991) Formate releases carbon dioxide/bicarbonate from thylakoid membranes. Naturwissenschaften, in press

Hansson Ö and Wydrzynski T (1990) Current perceptions of Photosystem II. Photosynth Res 23: 131–162

Harris EH (1988) The *Chlamydomonas* sourcebook: A comprehensive guide to biology and laboratory use. Academic Press, Inc., San Diego. ISBN 0–12–3268880–x (QK569.C486H37)

Havaux M, Strasser RJ and Greppin H (1991) A theoretical and experimental analysis of the q_P and q_N coefficients of chlorophyll fluorescence quenching and their relation to photochemical and non-photochemical events. Photosynth Res 27: 41–55

Johanningmeir U, Bodner U and Wildner GF (1987) A new mutation in the gene coding for the herbicide-binding protein in *Chlamydomonas*. FEBS Lett 211: 221–224

Karapetyan NV, Strasser R and Böger P (1983) Variable fluorescence and fluorescence spectra of algae after herbicide-induced pigment bleaching. Z Naturforsch 38C: 556–562

Michel H and Deisenhofer J (1988) Relevance of the photosynthetic reaction center from purple bacteria to the structure of photosystem II. Biochemistry 27: 1–7

Robinson H, Eaton-Rye JJ, van Rensen JJS and Govindjee (1989) The effects of bicarbonate depletion and formate incubation on the kinetics of oxidation-reduction reactions of the photosystem II quinone acceptor complex. Z Naturforsch 39C: 382–385

Shopes R, Blubaugh DJ, Wraight CA and Govindjee (1989) Absence of a bicarbonate-depletion effect in electron transfer between quinones in chromatophores and reaction centers of *Rhodobacter sphaeroides*. Biochim Biophys Acta 974: 114–118

Spalding MH, Critchley C, Govindjee and Ogren WL (1984) Influence of carbondioxide concentration during growth on fluorescence induction characteristics of the green alga *Chlamydomonas reinhardtii*. Photosynth Res 5: 169–176

Strasser RJ and Sironval C (1972) Induction of photosystem II activity in flashed leaves. FEBS Lett 28: 56–60

Sundby C, Larsson UK and Henrysson T (1989) Effects of bicarbonate on thylakoid protein phosphorylation. Biochim Biophys Acta 975: 277–282

Trebst A (1987) The three-dimensional structure of the herbicide binding niche on the reaction center polypeptides of photosystem II. Z Naturforsch 42C: 742–750

van Rensen JJS, Tonk WJM and de Bruijn SM (1988) Involvement of bicarbonate in the protonation of the secondary quinone electron acceptor of photosystem II via the non-haem iron of the quinone acceptor complex. FEBS Lett 226: 347–351

Vermaas WFJ and Govindjee (1981) The acceptor side of photosystem II in photosynthesis. Photochem Photobiol 34: 775–793

Whitmarsh J and Togaski R (1986) Multidisciplinary research in photosynthesis: A case history based on *Chlamydomonas*. Photosynth Res 10: 415–422