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Characterization of new strains of nonphotosynthetic mutants of *Chlamydomonas reinhardtii* II. Quinones and cytochromes *b*-559, *b*-563 and *c*-553 in twelve mutants having impaired Photosystem II function

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Twelve new strains of nonphotosynthetic mutants of *Chlamydomonas reinhardtii* having impaired functioning of Photosystem II were studied with respect to their quinone and chloroplastic cytochrome contents and to various photooxidation reactions of cytochromes *b*-559 and *c*-553. The quinones were analyzed by chromatography, cytochromes *b*-563 and *c*-553 were measured spectrophotometrically after solubilization by Triton X-100, and cytochrome *b*-559 was studied by means of low-temperature difference spectra.

None of these mutants showed a great deficiency of plastoquinone A, ubiquinone Q9, cytochrome *b*-563 or cytochrome *c*-553. But all lacked an ascorbate-reducible pool of cytochrome *b*-559 photooxidizable at 77°K. In spite of this deficiency, five mutants (*Fl* 18, *Fl* 29, *Fl* 47, *Fl* 50, *Fl* 59) showed an appreciable photooxidation of cytochrome *b*-559 in the presence of FCCP at room temperature. The other strains performed only weak cytochrome *b*-559 photooxidation in the presence of FCCP, DCMU and DBMIB or *p*-benzoquinone (*Fl* 39, *Fl* 42, *Fl* 52, *Fl* 54, *Fl* 57, *Fl* 60); in the mutant *Fl* 33, no cytochrome photooxidation was observed.

These results pointed out that the pool of ascorbate-reducible cytochrome *b*-559 photooxidizable at 77°K is different from the pool photooxidizable in the presence of FCCP at room temperature.

Key words: *Chlamydomonas reinhardtii* — Chloroplastic cytochromes — Nonphotosynthetic mutants — Photosystem II — Plastoquinone.

Various electron carriers (plastoquinone, cytochromes *b*-559, *b*-563 and *c*-553, plastocyanin, ferredoxin) participate in photosynthetic electron transport (see ref. *16*). Despite many papers on the subject, the role of cytochrome *b*-559 in photosynthesis and its localization in the membrane have not yet been clearly elucidated.

Abbreviations: A—F, ascorbate-reduced *minus* ferricyanide-oxidized; CCCP, carbonylcyanide-*m*-chlorophenylhydrazone; C-550, pigment indicator of the state of Photosystem II centers, responsible for absorbance changes around 546–550 nm; D—A, dithionite-reduced *minus* ascorbate-reduced; D—F, dithionite-reduced *minus* ferricyanide-oxidized; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; HQ—F, hydroquinone-reduced *minus* ferricyanide-oxidized; P700, chlorophyll *a* holochrome, active pigment in Photosystem I; SDS, sodium dodecylsulfate.

One of the features of this cytochrome *b*-559 is that it exists in several forms with different redox potentials: a high-potential hydroquinone-reducible form which is unstable and can be transformed by various factors into the other forms, a middle-potential form that is ascorbate-reducible but not hydroquinone-reducible, and a low-potential form that is only dithionite-reducible. The cytochrome *b*-559 reactions are mostly Photosystem II-dependent and several possible roles have been proposed for this cytochrome such as a role as an electron carrier in a cycle around Photosystem II or in the main chain between the two photosystems, as a proton carrier or as an active agent in the water-splitting process. But what occurs really in vivo has not yet been clearly elucidated: for instance, Photosystem II-related photooxidations of cytochrome *b*-559 were observed only under artificial conditions (low temperature, presence of CCCP or FCCP, Tris-washed chloroplasts) which generally inactivated, at least partially, the natural water-splitting system; in addition, many experimental treatments are known to modify the potential of cytochrome *b*-559 (see reviews *4*, *6*).

Epel et al. (*7*, *8*), studying chloroplast fragments of various nonphotosynthetic mutants of *Chlamydomonas reinhardtii*, pointed out the existence of two functionally distinct pools of ascorbate-reducible cytochrome *b*-559, both acting in the water-splitting oxygen-evolving apparatus. One of these pools, photooxidizable at 77°K, was missing in the high-fluorescent mutants but present in the low-fluorescent mutants. In our laboratory, we studied the cytochrome *b*-559 photooxidation in chloroplast fragments of the wild type and of three nonphotosynthetic mutants (*Fl* 5 devoid of P700, *Fl* 9 and *Fl* 15 deficient in cytochromes *b*-563 and *c*-553) of *C. reinhardtii* in the presence of antimycin or FCCP. We observed that: (1) two types of photooxidation occurred which were either Photosystem II-dependent or Photosystem I-dependent, and (2) part of the cytochrome which was oxidized by Photosystem II was hydroquinone-reducible while the other part was ascorbate-reducible (*14*, *23*).

Several new strains of nonphotosynthetic mutants of *C. reinhardtii* have been recently isolated in our laboratory and their properties concerning pigment contents, photochemical activities (Hill and Mehler reactions), chlorophyll fluorescence induction kinetics and SDS-polyacrylamide gel electrophoresis of the chlorophyll-protein complexes CP I and CP II, have been described in a preceding paper (*10*). Twelve of these strains showed impaired functioning of the Photosystem II: eight mutants (*Fl* 39, *Fl* 42, *Fl* 50, *Fl* 52, *Fl* 54, *Fl* 57, *Fl* 59, *Fl* 60) did not emit any variable chlorophyll fluorescence and their chloroplast fragments could not carry out any DCIP photoreduction, while four mutants (*Fl* 18, *Fl* 29, *Fl* 33, *Fl* 47) showed some variable fluorescence and their chloroplast fragments performed weak Hill reactions with DCIP. Therefore we thought it would be interesting to investigate the cytochrome *b*-559 contents and the possible occurrence of some cytochrome photooxidation reactions in these various new mutants. In order to more precisely examine the properties of these strains, their plastoquinone A, ubiquinone Q9, cytochrome *b*-563 and cytochrome *c*-553 contents were also measured. The results indicate clearly that all the examined mutants lacked the pool of ascorbate-reducible cytochrome *b*-559 photooxidizable at 77°K. Nevertheless, five of them performed the photooxidation of another ascorbate-reducible cytochrome *b*-559 pool, in presence of FCCP at room temperature.

Materials and methods

The characteristics of the new strains of mutants *Fl...* of *C. reinhardtii*, recently isolated in our laboratory, and of the wild type have been described in a preceding paper (*10*). The mutant *ac*-115 from Dr. R. P. Levine's laboratory (Harvard University, Cambridge) lacks functional cytochrome *b*-559 and does not show any Photosystem II activity (*13*, *19*, *20*).

The algae were grown in light in Tris-acetate-phosphate medium (*15*) as previously reported (*11*, *12*). To prepare chloroplast fragments, the cells suspended in a buffer of 0.01 M K phosphate, 0.02 M KCl and 2.5×10^{-3} M $MgCl_2$ (pH 7.5), were disrupted for 30 sec in a sonic oscillator. Next, the chloroplast fragments were separated by two successive centrifugations at $480 \times g$ for 6 min and at $20{,}000 \times g$ for 15 min, then suspended in fresh buffer. The chlorophyll contents were measured according to MacKinney (*21*) and Arnon (*2*).

The quinones were extracted from whole cells with acetone, chromatographed on silicic acid columns then on silica gel thin layers, and spectrophotometrically titrated as previously described (*11*). Cytochromes *b*-563 and *c*-553 were solubilized from chloroplast fragments with the detergent Triton X-100 following the method of Hind and Nakatani (*18*), then measured by difference spectrophotometry as previously indicated (*22*).

The low-temperature difference spectra of chloroplast fragments were measured using an Aminco-Chance spectrophotometer in split beam mode with an accessory device including Bonner's cells and liquid nitrogen containing Dewar's vessel. When necessary, the frozen preparations were illuminated in the spectrophotometer compartment by a microscope lamp through a light guide. For the approximate calculation of the respective absorbances due to cytochrome *b*-559 and cytochrome *c*-553 in the ascorbate-reduced *minus* ferricyanide-oxidized spectra (A—F), the following equations were used:

$$A_{557(\text{cyt. } b\text{-}559)} = 1.12\, A_{557} - 0.24\, A_{553}$$
$$A_{553(\text{cyt. } c\text{-}553)} = 1.12\, A_{553} - 0.58\, A_{557}$$

where $A_{557(\text{cyt. } b\text{-}559)}$ represents the contribution of cytochrome *b*-559 to the total absorbance at 557 nm (A_{557}) and $A_{553(\text{cyt. } c\text{-}553)}$ the contribution of cytochrome *c*-553 to the total absorbance at 553 nm (A_{553}). These equations had been calculated using the absorbance ratios $A_{553}/A_{557}=0.52$ for cytochrome *b*-559 and $A_{557}/A_{553}=0.21$ for cytochrome *c*-553 from difference spectra obtained for the cytochrome *c*-553-deficient mutant *Fl* 15 of *C. reinhardtii* and for purified cytochrome *c*-553 (*11*).

At room temperature, the photooxidation of the cytochromes *b*-559 and *c*-553 in whole cells was measured as previously described (*23*) using the dual wavelength mode of the Aminco-Chance spectrophotometer.

Results

Quinones

During our previous studies, only the chloroplastic plastoquinone A and the

Table 1 *Plastoquinone A, ubiquinone Q9, cytochrome* b-*563 and cytochrome* c-*553 contents of the wild type and mutant strains of* C. reinhardtii

Strain	Quinone				Cytochrome	
	Plastoquinone A		Ubiquinone Q9		*b*-563	*c*-553
	(a)	(b)	(c)	(d)	(e)	(f)
Wild type	6.9	(0.32)	2.5	(0.12)	3.2	1.8
Fl 18	9.0	(0.52)	3.3	(0.19)	2.3	1.8
Fl 29	7.9	(0.41)	2.9	(0.15)	3.2	2.2
Fl 33	6.0	(0.30)	2.3	(0.12)	1.8	1.0
Fl 39	10.0	(0.43)	2.4	(0.10)	2.7	1.7
Fl 42	15.0	(0.62)	8.1	(0.34)	2.4	1.6
Fl 47	30.0	(0.63)	9.7	(0.20)	2.1	1.4
Fl 50	13.5	(0.55)	2.6	(0.11)	2.5	1.2
Fl 52	8.9	(0.53)	3.3	(0.20)	2.6	1.3
Fl 54	11.0	(0.50)	3.5	(0.16)	2.7	1.3
Fl 57	10.0	(0.45)	3.0	(0.12)	2.4	1.6
Fl 59	7.6	(0.39)	2.3	(0.12)	2.4	1.8
Fl 60	6.0	(0.31)	2.2	(0.11)	2.2	1.3

Contents: (a), (c), (e), (f): μmoles/mmole of chlorophyll *a*+*b*; (b), (d), numbers in parentheses: μmoles/g of dry matter. The plastoquinone and the ubiquinone were extracted from whole cells with acetone; the cytochromes *b*-563 and *c*-553 were solubilized from chloroplast fragments with Triton X-100 (see **Materials and methods**).

mitochondrial ubiquinone Q9 were detected in cells of the wild type and the three mutants *Fl* 5, *Fl* 9, *Fl* 15 of *C. reinhardtii* (*11*). The quinone contents of the new mutants strains and of the wild type are summarized in Table 1. These results indicate clearly that none of the twelve new mutants analyzed showed any deficiency in plastoquinone A or ubiquinone Q9; indeed the various values obtained for the mutants are close to the values of the wild type. It is difficult to attach any great importance to the differences which appeared between the contents of the various strains because, as previously observed (*11*), the quinone content of the same strain may vary largely from one harvested culture to another.

Cytochromes b-*563 and* c-*553*

The cytochrome *b*-563 and cytochrome *c*-553 contents of chloroplast fragments, which were measured after solubilization in Triton X-100, are also given in Table 1. The values concerning the wild type are of the same magnitude as those we obtained in a previous work (2.7 nmoles of cytochrome *b*-563 and 2.1 nmoles of cytochrome *c*-553 per μmole of chlorophyll *a*+*b*) (*11*, *22*). This corroborates average contents of about 1 mole of cytochrome *b*-563 per 340 moles of chlorophyll and 1 mole of cytochrome *c*-553 per 500 moles of chlorophyll in the wild type of *C. reinhardtii*.

Though the contents of some strains, like *Fl* 33, were lower than those of the wild type, all the examined mutants clearly appeared to have both cytochromes *b*-563 and *c*-553. None of them showed any great deficiency equivalent to the previously observed deficiencies of the mutants *Fl* 9 and *Fl* 15 of *C. reinhardtii* [less than 19% of cytochrome *b*-563, only traces of cytochrome *c*-553 (*22*)].

Cytochrome b-*559*: *difference spectra at 77°K*

The total cytochrome *b*-559 contents of Triton X-100-treated chloroplast fragments of *C. reinhardtii*, could not be reproducibly measured because most of this cytochrome remained masked or had been damaged (see ref. *22*). In addition, no satisfactory results were obtained from acetone depigmented chloroplast fragments. Therefore, in order to more precisely examine the cytochrome *b*-559 contents of the various mutants, several difference spectra were measured at 77°K using chloroplast fragments. In the wild type (Fig. 1), the D—F (dithionite-reduced *minus* ferricyanide-oxidized) spectrum, which corresponds to the sum of the reduced *minus* oxidized spectra of all the different chloroplast cytochromes, showed a very distinct maximum at 557 nm and a small shoulder around 561 nm. The D—A (dithionite-reduced *minus* ascorbate-reduced) spectrum showed a broad maximum around 559 nm, indicating the presence of both low potential (only dithionite-reducible) cytochrome *b*-559 (peak at 557 nm) and cytochrome *b*-563 (peak at 561 nm). On these D—F and D—A spectra, negative peaks around 547 nm were indicative of the presence of the Photosystem II pigment C-550, which is also dithionite-reducible. And the HQ—F (hydroquinone-reduced *minus* ferricyanide-oxidized) spectrum showed only the presence of cytochrome *c*-553 (peak at 553 nm and shoulder at 548 nm) which is the sole hydroquinone-reducible one in these preparations. The A_{dark}—F_{dark} (dark ascorbate-reduced *minus* dark ferricyanide-oxidized) spectrum, which showed a maximum around 555–556 nm and a shoulder around 548 nm, indicated

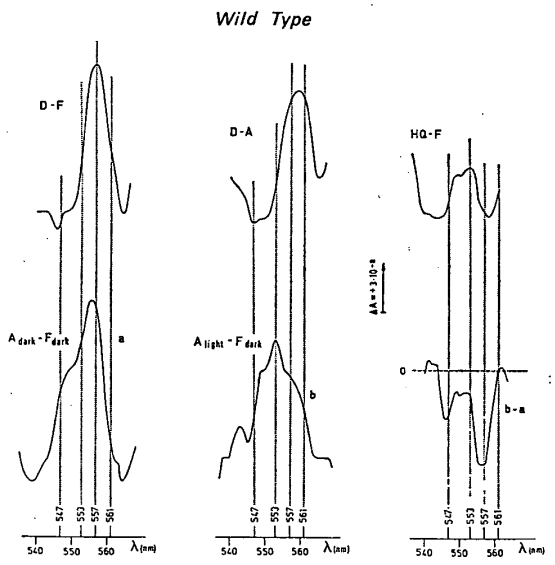

Fig. 1. *Low-temperature difference spectra of chloroplast fragments of the wild type of* C. reinhardtii. D—F: dithionite-reduced *minus* ferricyanide-oxidized; D—A: dithionite-reduced *minus* ascorbate-reduced; HQ—F: hydroquinone-reduced *minus* ferricyanide-oxidized; A—F: ascorbate-reduced *minus* ferricyanide-oxidized. The reactives (0.04 M Na ascorbate, 0.004 M K ferricyanide or few cristals of Na dithionite or of hydroquinone) were added to the chloroplast fragments which had been suspended in 0.01 M phosphate buffer (pH 7.5), at concentrations corresponding to 250 μg of chlorophyll $a+b$/ml, then the suspensions were frozen in Bonner's cells having a 2-mm light path using liquid nitrogen. A_{dark}, A_{light}: after the recording of spectrum a, the same frozen ascorbate-reduced sample was preilluminated in the spectrophotometer compartment then spectrum b was measured. Except for b, all spectra were measured with nonpreilluminated samples.

the presence of ascorbate-reducible cytochrome *b*-559 and of cytochrome *c*-553. But on the $A_{light}-F_{dark}$ (light ascorbate-reduced *minus* dark ferricyanide-oxidized) spectrum (for which the ascorbate-reduced sample had been preilluminated at low temperature), the maximum appeared at 553 nm and the cytochrome *b*-559 contribution was lowered to a shoulder around 557 nm. This indicated that part of the cytochrome *b*-559 had become oxidized in light; indeed, the light *minus* dark difference between both these A—F spectra (Fig. 1, b—a) clearly showed two negative peaks respectively around 546–547 nm and around 557 nm, which gave evidence of both classical Photosystem II-dependent low-temperature reactions: the C-550 photoreduction and the cytochrome *b*-559 photooxidation.

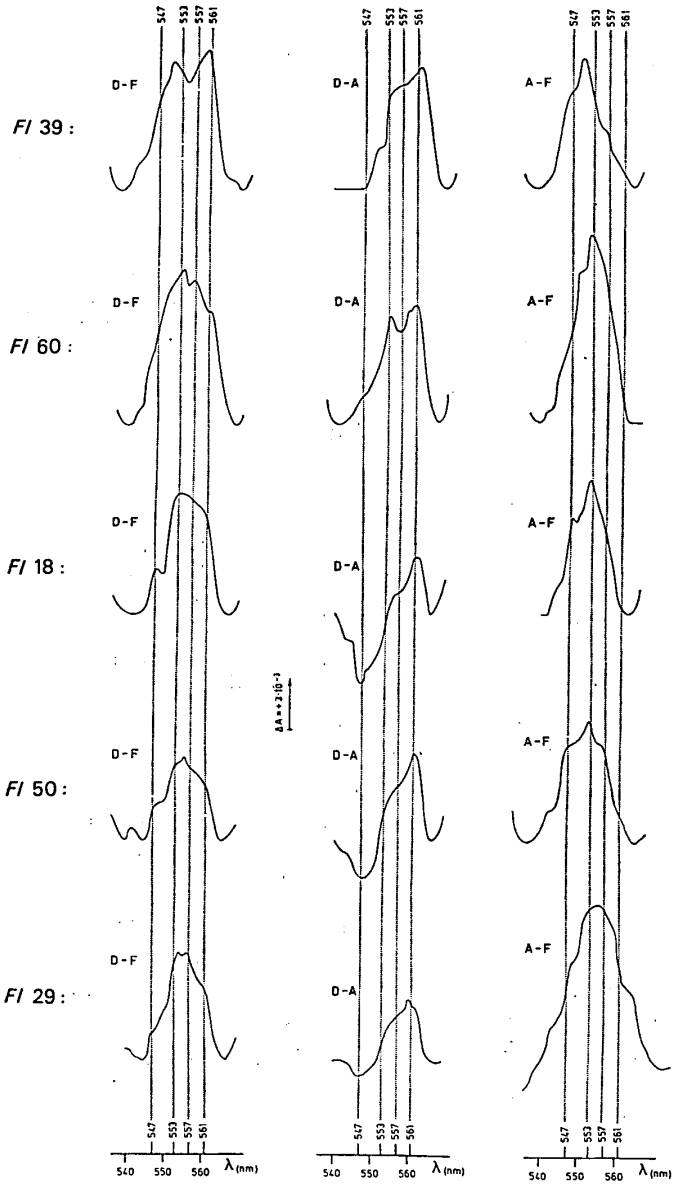

Fig. 2. *Low-temperature difference spectra of chloroplast fragments of five mutants of* C. reinhardtii. See the legend of Fig. 1 for abbreviations and experimental conditions. All spectra were measured with nonpreilluminated samples. For A—F spectra, notice that the relative size of the shoulders around 557 nm increased from the upper part (*Fl* 39) to the lower part (*Fl* 29) of this figure.

Difference spectra of five typical mutants are shown in Fig. 2. We can see easily that all these mutants contained less cytochrome *b*-559 than the wild type: on the D—F spectra, only a dip (*Fl* 39) or shoulders (other mutants) were observed instead of the peak which was obtained in the wild type case (Fig. 1); on the D—A spectra, the maxima were shifted to near 561 nm. Nevertheless, the shoulder around 557 nm on the D—A spectra (Fig. 2, except in the case of *Fl* 60) indicated the presence of low-potential dithionite-reducible cytochrome *b*-559 in these strains. Also note that on the *Fl* 18, *Fl* 50 and *Fl* 29 D—A spectra, little negative peaks around 547 nm showed the presence of reduced C-550. But the most interesting information came from the A—F spectra. Indeed for all the mutants, these A—F spectra (Fig. 2) measured using samples kept in the dark, were of the same type as the $A_{light}-F_{dark}$ spectrum of the wild type (Fig. 1, b): maxima around 553 nm and only shoulders around 557 nm. And no significant differences were observed when A—F spectra of the mutants were measured using preilluminated ascorbate-reduced preparations (not shown). Therefore it appeared that all the mutants

Table 2 *Evaluation of absorbances due to cytochrome* b-*559 in low-temperature A—F difference spectra of chloroplast fragments of the wild type and mutant strains of* C. reinhardtii

Strain	Absorbance $A_{557(cyt.\ b-559)}$ ($\times 10^3$)	Absorbance ratio	
		$\frac{A_{557}}{A_{553}}$	$\frac{A_{557(cyt.\ b-559)}}{A_{553(cyt.\ c-553)}}$
Wild type: dark	8.7	1.3	3.1
light	4.6	0.9	1.3
ac-115[a]	2.1	0.5	0.4
Fl 18	3.6	0.6	0.6
Fl 29	5.3	0.8	1.3
Fl 33	0.8	0.4	0.3
Fl 39	1.9	0.4	0.3
Fl 42	3.8	0.7	0.8
Fl 47	5.7	0.9	1.2
Fl 50	5.6	0.8	1.1
Fl 52	5.2	1.0	1.6
Fl 54	4.7	0.7	0.7
Fl 57	5.9	0.8	1.0
Fl 59	3.5	0.7	0.8
Fl 60	4.9	1.0	0.9

A_{553}, A_{557}: absorbance at 553 nm and at 557 nm directly measured on the spectra; $A_{553(cyt.\ c-553)}$, $A_{557(cyt.\ b-559)}$: calculated absorbances due respectively to the cytochrome *c*-553 at 553 nm and to the cytochrome *b*-559 at 557 nm. Dark: values corresponding to $A_{dark}-F_{dark}$ spectra measured with nonpreilluminated samples; light: values corresponding to $A_{light}-F_{dark}$ spectra measured with ascorbate-reduced samples which had been preilluminated (see Fig. 1); all values relative to the mutants are for $A_{dark}-F_{dark}$ spectra. For the measurements of the absorbance values on the original spectra, the isobestic points at 570–575 nm were used as references. The absorbance values attributable respectively to cytochromes *b*-559 and *c*-553 were approximately calculated using the equations given in **Methods**; they correspond to algal suspensions containing 250 μg of chlorophyll *a*+*b*/ml which were frozen in Bonner's cells having a 2-mm light path.

[a] Levine's mutant strain.

lacked the pool of ascorbate-reducible cytochrome *b*-559 photooxidizable at 77°K. In addition, no C-550 photoreduction was observed. Nevertheless all were not identically deficient. On the A—F spectra of Fig. 2, the size of the shoulders around 557 nm was significantly smaller for strains *Fl* 39 and *Fl* 60 than strains *Fl* 50 and *Fl* 29.

The spectra of the other examined mutants were like those shown in Fig. 2. All were deficient in ascorbate-reducible low temperature-photooxidizable cytochrome *b*-559 and the extent of their total deficiencies in cytochrome *b*-559 varied depending on the strain. In an attempt to quantify these deficiencies, we have indicated in Table 2 absorbance values and absorbance ratios relative to cytochrome *b*-559 calculated approximately from the spectra A—F for the different strains. In the case of each mutant, the absorbance attributable to the cytochrome *b*-559, $A_{557(\text{cyt. } b\text{-}559)}$, and the $A_{557(\text{cyt. } b\text{-}559)} / A_{553(\text{cyt. } c\text{-}553)}$ ratio, which corresponds to the ascorbate-reducible cytochrome *b*-559/cytochrome *c*-553 ratio, were close to the values obtained for illuminated preparations of the wild type.

Cytochrome b-*559*: *photooxidation in the presence of FCCP at room temperature*

We have previously studied at room temperature the photoxidation of cytochrome *b*-559 in the presence of FCCP in whole cells and chloroplast fragments of the wild type and three non-photosynthetic mutants (*Fl* 5, *Fl* 9, *Fl* 15) of *C. reinhardtii* (*14*, *23*). Two types of cytochrome *b*-559 photooxidation were observed: a Photo-

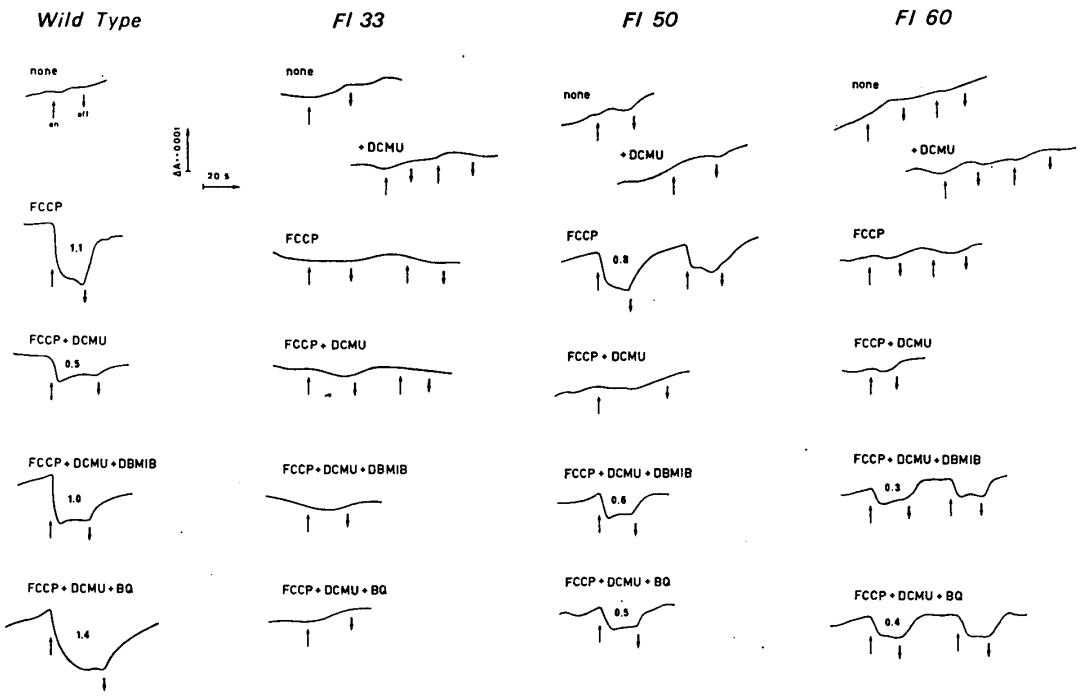

Fig. 3. *Photooxidation of cytochrome* b-*559 in whole cells of the wild type and three mutants of* C. reinhardtii *at room temperature.* The cells were suspended in a mixture of 0.01 M phosphate buffer (pH 7.5) and 5% dextran T 80, at concentrations corresponding to 100 μg of chlorophyll $a+b$/ml. Actinic light: 690–730 nm at half transmission of the filter, 20 $W \cdot m^{-2}$. Analytical light: 559 nm (reference λ: 570 nm). Concentrations of the reactives: 1.2×10^{-5} M DCMU, 1.2×10^{-4} M FCCP, 4.8×10^{-5} M DBMIB, 1.2×10^{-4} M *p*-benzoquinone (BQ). ↑: light on; ↓: light off. The numbers indicate the total absorbance changes ($\times 10^3$) for 100 μg of chlorophyll $a+b$/ml.

system II-dependent photooxidation which occurred in the presence of FCCP and was inhibited by DCMU but occurred again in the presence of FCCP, DCMU and DBMIB, and a Photosystem I-dependent photooxidation which was observed in whole cells in the presence of FCCP, DCMU and *p*-benzoquinone. In the present work, we examined which cytochrome *b*-559 photooxidations occurred in whole cells of the new mutant strains. The results are given in Table 3 and the three types of responses observed are illustrated in Fig. 3. Five mutants, *Fl* 18, *Fl* 29, *Fl* 47, *Fl* 50 and *Fl* 59, carried out all the photooxidations described above but their amplitudes were weaker than in the wild type. Six other mutants, *Fl* 39, *Fl* 42, *Fl* 52, *Fl* 54, *Fl* 57 and *Fl* 60, did not perform any cytochrome *b*-559 photooxidation in the presence of FCCP alone but carried out weak photooxidations when exogenous carriers like DBMIB or *p*-benzoquinone were added. These latter mutants behaved like Levine's mutant *ac*-115. In the case of strain *Fl* 33, no cytochrome *b*-559 photooxidation was observed.

The values relative to the photooxidation of cytochrome *c*-553 by untreated cells are indicated in Table 3. The cytochrome *c*-553 photooxidation, a Photosystem I-dependent reaction, was observed for all the mutants except three. In mutant *Fl* 47, cytochrome *c*-553 reduction instead of oxidation occurred in light and

Table 3 *Photooxidation of cytochromes* b-*559 and* c-*553 in whole cells of the wild type and mutant strains of* C. reinhardtii

Strain	Cytochrome *b*-559						Cytochrome *c*-553
	No add.	+DCMU	+FCCP	+FCCP +DCMU	+FCCP +DCMU +DBMIB	+FCCP +DCMU +BQ	No add.
Wild type	0	0	0.50	0.21	0.46	0.62	0.22
ac-115[a]	0	0	0.00	0.00	0.12	0.12	0.22
Fl 18	0	0	0.17	0.08	0.21	0.15	0.12
Fl 29	0	0	0.19	0.08	0.25	0.25	0.22
Fl 33	0	0	0.00	0.00	0.00	0.00	0.00
Fl 39	0	0	0.00	0.00	0.17	0.23	0.00
Fl 42	0	0	0.00	0.00	0.12	0.12	0.12
Fl 47	0	0	0.15	0.00	0.23	0.08	−0.34[b]
Fl 50	0	0	0.35	0.00	0.27	0.23	0.18
Fl 52	0	0	0.00	0.00	0.19	0.23	0.25
Fl 54	0	0	0.00	0.00	0.19	0.27	0.11
Fl 57	0	0	0.00	0.00	0.15	0.27	0.12
Fl 59	0	0	0.23	0.00	0.19	0.15	0.31
Fl 60	0	0	0.00	0.00	0.15	0.19	0.11

Results: μmoles of oxidized cytochrome/mmole of chlorophyll $a+b$. The cells were suspended in a mixture of 0.01 M phosphate buffer (pH 7.5) and 5% dextran T 80 at concentrations corresponding to 100 μg of chlorophyll $a+b$/ml. Actinic light: 690–730 nm at half transmission of the filter, 20 $W \cdot m^{-2}$. Analytical lights: 559 nm for cytochrome *b*-559, 553 nm for cytochrome *c*-553 (reference λ: 570 nm). Concentrations of the reactives: 1.2×10^{-5} M DCMU, 1.2×10^{-4} M FCCP, 4.8×10^{-5} M DMBIB, 1.2×10^{-4} M *p*-benzoquinone (BQ). No add.: no addition.

[a] Levine's mutant strain.

[b] A photoreduction was observed in place of photooxidation.

was inhibited by DCMU (not shown). We also observed that in this mutant with very weak Photosystem II activity, Photosystem I activity also appeared weaker than those of the other strains. This weaker Photosystem I activity was corroborated by the very small amount of cytochrome *b*-559 photooxidized in the presence of FCCP+DCMU+*p*-benzoquinone (Table 3). Therefore the photooxidation of cytochrome *c*-553 by the Photosystem I seems to occur more slowly than its reduction by the Photosystem II. Finally, in the cases of two strains, *Fl* 33 and *Fl* 39, no cytochrome *c*-553 photoreaction was observed. In these mutants with a functioning Photosystem I, cytochrome *c*-553 may be fully oxidized in the dark; also note that the cytochrome *c*-553 content of mutant *Fl* 33 was relatively small (Table 1).

Discussion

None of the twelve mutants of *C. reinhardtii* examined here showed any great deficiency of plastoquinone, cytochrome *b*-563 or cytochrome *c*-553. Therefore the weakness or total absence of Photosystem II-related photosynthetic activity, previously observed in these strains (*10*), cannot be imputed to the lack of these electron carriers. Besides, cytochromes *b*-563 and *c*-553 act in the electron transport systems towards or around Photosystem I and mutants devoid of both these cytochromes, like *Fl* 9 and *Fl* 15, have Photosystem II-dependent activities (*12*, *13*). Nevertheless the fact that the total plastoquinone contents of these new mutants were comparable to the wild type content does not allow exclusion of the possibility that some moles of plastoquinone, which are normally closely bound to active Photosystem II centers, are not functional in certain mutants because of eventual membrane structure defects.

All the strains which had impaired Photosystem II function showed anomalies concerning cytochrome *b*-559. According to the results obtained by Epel et al. (*7*, *8*) for various low-fluorescent and high-fluorescent nonphotosynthetic mutants of *C. reinhardtii*, there is one mole of low temperature-photooxidable cytochrome *b*-559 per mole of C-550, i.e., half the ascorbate-reducible cytochrome *b*-559 present in the wild type. In the same way in higher plant chloroplasts, various authors (*1*, *3*, *9*, *24*) estimated that the amount of cytochrome *b*-559, which was photooxidized at 77°K, corresponded to half the total cytochrome *b*-559 present. The results we obtained show that all the twelve new mutants of *C. reinhardtii* lacked this ascorbate-reducible and low temperature-photooxidizable pool of cytochrome *b*-559. These new mutants are for this reason comparable to the high-fluorescent mutants described by Epel and Butler (*7*).

In spite of their deficiencies in low temperature-photooxidizable cytochrome *b*-559, five mutants clearly performed photooxidation of cytochrome *b*-559 in the presence of FCCP. We have shown elsewhere that the cytochrome *b*-559 photooxidized in the presence of FCCP in chloroplast fragments of *C reinhardtii* was ascorbate-reducible and even partly hydroquinone-reducible (*14*, *23*). This pool of cytochrome was also detectable at low temperature (Fig. 2, Table 2).

The other mutants carried out weak cytochrome *b*-559 photooxidation only if exogenous electron carriers (DBMIB or *p*-benzoquinone) were added and no C-550 photoreduction at 77°K was seen in these mutants, though this latter pigment, chemically reduced by dithionite, had been detected in several strains. Both these

facts are indicative of anomalies of the electron transfer around Photosystem II.

The ascorbate-reducible cytochrome *b*-559 missing in these mutants did not seem to have been transformed into a low-potential form. Indeed, the D—F difference spectra showed that all these mutants had lower cytochrome *b*-559 total contents than the wild type and on the D—A difference spectra, none appeared to have more dithionite-reducible cytochrome *b*-559 than the wild type.

We have previously pointed out that FCCP or antimycin were necessary for the occurrence of cytochrome *b*-559 photooxidation in chloroplast fragments or whole cells of *C. reinhardtii*. We have also shown that cytochrome *b*-559 photooxidation occurred at a FCCP concentration (1.2×10^{-5} M) which only caused slight inhibition (less than 20%) of the oxygen evolution of the wild type (*14*, *23*). In this present work, we observed that in five mutants, which showed no Photosystem II activity (*Fl* 50, *Fl* 59) or very weak activity (*Fl* 18, *Fl* 29, *Fl* 47), FCCP allowed the occurrence of an appreciable, reversible and DCMU-sensitive cytochrome *b*-559 photooxidation. These facts indicate that the action of FCCP on cytochrome *b*-559 is not directly related to an electron transfer inhibition and raise a question concerning the mode of FCCP action on the chloroplast membrane. Also the occurrence of cytochrome *b*-559 photooxidation in the presence of FCCP does not seem to be essentially due to a change in the potential of this cytochrome; indeed, as previously observed, the cytochrome *b*-559 photooxidized in the presence of FCCP in chloroplast fragments of *C. reinhardtii* was still partly hydroquinone-reducible (*14*, *23*). But it is possible that FCCP modified the structural properties of the chloroplast membrane and therefore the cytochrome *b*-559 accessibility to some electron donors and acceptors. Such modifications would facilitate the trapping of positive charges by the cytochrome *b*-559 and the functioning of a cyclic transfer around Photosystem II, even in mutants with no apparent Photosystem II activity. Cramer et al. (*5*) have suggested upon the ground of measurements carried out with *Escherichia coli* (*17*), that FCCP may act by increasing the thylakoid membrane microviscosity.

In conclusion, these results with twelve nonphotosynthetic mutants of *C. reinhardtii* confirmed that there is a close relation between impaired Photosystem II function and deficiencies in cytochrome *b*-559. They pointed out that the ascorbate-reducible cytochrome *b*-559 pool photooxidizable at 77°K (missing in all the mutants) is different from the pool which is photooxidizable at room temperature in the presence of FCCP (present in five mutants). Further experiments are in progress to understand how FCCP acts on the chloroplast membrane allowing photooxidation of cytochrome *b*-559 by Photosystem II.

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