*Biochimica et Biophysica Acta*, 637 (1981) 473–480
Elsevier/North-Holland Biomedical Press



BBA 48143

# GEL ELECTROPHORESIS OF CHLOROPLAST MEMBRANES OF MUTANTS OF *CHLAMYDOMONAS REINHARDII* WHICH HAVE IMPAIRED PHOTOSYSTEM II FUNCTION AND LACK PHOTOSYNTHETIC CYTOCHROMES

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(Received March 17th, 1981)

*Key words: Photosystem II; Chloroplast membrane; Chlorophyll-protein complex; Cytochrome; (Chlamydomonas reinhardii)*

**Photosystem (PS) II-enriched particles or chloroplast fragments of the wild type and of three nonphotosynthetic mutants of *Chlamydomonas reinhardii*, which lack chloroplast cytochromes, were analyzed by lithium dodecyl sulfate polyacrylamide gel electrophoresis at 4°C to locate which chlorophyll complexes and which proteins are associated with cytochrome *b*-559. Two mutants, *Fl* 39 and *Fl* 50, have previously been shown to contain, respectively, 3.6- and 2.7-times less hydroquinone-reducible high-potential cytochrome *b*-559 than the wild type. They have impaired PS II functions. In the presence of ADRY agents: carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP), 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene (ANT 2p) or 2-(3,4,5-trichloro)-anilino-3,5-dinitrothiophene (ANT 2s), *Fl* 50 carried out photo-oxidation of cytochrome *b*-559 with half the amplitude of that of the wild type. No photo-oxidation was observed with *Fl* 39. We show here that in both these mutants chlorophyll-protein complexes CP III, CP IV and CP V were missing. There were traces of the corresponding apoproteins (45 000, 42 000 and 33 000 daltons, respectively) in *Fl* 50 but none in *Fl* 39. In addition, a 19 000 dalton protein was missing in *Fl* 39 and present in a very small amount in *Fl* 50. In another mutant, *Fl* 9, previously characterized as lacking both cytochromes *b*-563 and *c*-553 with a normal cytochrome *b*-559 content, CP III–CP V and the 19 000 dalton protein were detected. CP I (110 000 daltons) and CP II (24 000 daltons) were present in all strains. These observations confirmed the close relationship between deficiencies in cytochrome *b*-559, lack of CP III and CP IV and anomalies in the photochemistry of PS II. They provided additional evidence that CP V and a 19 000 dalton protein are also involved in this PS II photochemistry. Staining of the gels with 3,3′,5,5′-tetramethylbenzidine and $H_2O_2$ allowed us to distinguish clearly four heme protein bands having peroxidase activity. Three of these bands (45 000, 42 000 and 19 000 daltons), which were shown in wild-type, *Fl* 39 and *Fl* 50 preparations but not in *Fl* 9, appeared related to cytochromes *b*-563 and *c*-553. The fourth heme protein (14 000 daltons) occurred in wild type and *Fl* 9 but was missing in *Fl* 39 and *Fl* 50; it appeared related to cytochrome *b*-559.**

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Abbreviations: ADRY, acceleration of the deactivation reactions of the water-splitting enzyme system Y of photosynthesis; ANT 2p, 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene; ANT 2s, 2-(3,4,5-trichloro)anilino-3,5-dinitrothiophene; C-550, pigment indicator of the state of PS II centers, responsible for absorbance changes around 550 nm; CP, chlorophyll-protein complex; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; TMBZ, 3,3′,5,5′-tetramethylbenzidine; LDS, lithium dodecyl sulfate; Chl, chlorophyll; PS, photosystem.

## Introduction

By means of polyacrylamide gel electrophoresis of thylakoid membranes of higher plants [1,2] or green algae [3–7], several authors were able to separate different chlorophyll-proteins complexes and different polypeptides. In particular, Delepelaire and Chua [8] isolated from *Chlamydomonas reinhardii* three chlorophyll-protein complexes, denoted CP III–CP V; they characterized two of them, CP III and CP

IV, which contain Chl *a* and carotenoids but no Chl *b* and which may be involved in the primary photochemistry of PS II.

The function and localization of cytochrome *b*-559 in the photosynthetic membrane have not yet been entirely elucidated, despite many works on the subject (see Ref. 9). Recently, in our laboratory, we isolated and characterized several nonphotosynthetic mutants of *C. reinhardii* which are deficient in cytochrome *b*-599 and have impaired PS II function [10, 11]. Therefore, it was of interest to analyze the thylakoid chlorophyll-protein complexes and the proteins of these mutants.

This paper shows the results we obtained with two mutants, *Fl* 39 and *Fl* 50. It appeared clearly that these mutants devoid of cytochrome *b*-559 were lacking CP III–CP V, and also a protein with an apparent molecular weight of 19 000. In addition, the use of a third mutant previously characterized, *Fl* 9, which lacks both cytochromes *b*-563 and *c*-553, allowed us to attribute to different cytochromes four heme proteins which were localized on the electrophoretograms.

## Materials and Methods

The characteristics of the mutants *Fl* 39 and *Fl* 50 of *C. reinhardii* and of the wild type have been described in preceding papers [10,11]; the mutant *Fl* 9 had been characterized previously [12,13]. Algae were grown in light, in Tris/acetate/phosphate medium [14], as indicated in Refs. 12 and 15.

The preparation of chloroplast fragments and the spectrophotometric measurement of cytochrome *b*-559 oxido-reduction at 559 nm, using an Aminco DW 2 dual-wavelength spectrophotometer, were performed as previously reported [12,16,17].

PS II-enriched particles were isolated according to Ref. 18. These particles and the chloroplast fragments were analyzed, without prior lipid extraction, by LDS-polyacrylamide gel electrophoresis, at 4°C in darkness, as indicated in Ref. 8 except that, for the resolving gel, 11% acrylamide was used in place of a linear gradient of acrylamide. For the determination of apparent molecular weights, well characterized standard proteins (Sigma) were used (molecular weight in parentheses): bovine serum albumin (68 000), egg albumin (45 000), chymotrypsinogen A (25 000), trypsinogen (24 000), trypsin inhibitor from soybean (21 000), myoglobin (17 000) and lysozyme (14 000); these proteins were stabilized by acetylation before electrophoresis, according to Ref. 19. For detection of the peroxidase activity of hemes, the staining of the gels with TMBZ and $H_2O_2$ was performed according to Refs. 20 and 21.

## Results

The mutants *Fl* 39 and *Fl* 50 of *C. reinhardii*, deficient in cytochrome *b*-559, showed no oxygen evolution, no variable chlorophyll fluorescence, and were unable to carry out, at 77 K, both the classical PS II reactions: the C-550 photoreduction and the cytochrome *b*-559 photo-oxidation [10,11]. Spectrophotometric titration of chemically oxidized then reduced chloroplast fragments indicated clearly that these mutants contained 2.7-times (*Fl* 50) and 3.6-times (*Fl* 39) lower amounts of hydroquinone-reducible high-potential cytochrome *b*-559, and respectively, 2.1- and 3.1-times less total ascorbate-reducible cytochrome than the wild type (Fig. 1 and Table I). Photo-oxidations of cytochrome *b*-559 were observed with cells at room temperature in the presence of three ADRY agents; FCCP, ANT 2p and ANT 2s. The values obtained are summarized in Table II. In the wild type, appreciable oxidation of cytochrome *b*-559 took place in the light, the amplitude of which were greater with FCCP than with ANT 2p or ANT 2s. These photo-oxidations appeared to be mainly PS II-dependent reactions. In the case of the mutant *Fl* 50, photo-oxidations were also clearly observed with the ADRY agents, but their amplitudes were 2-times smaller than for the wild type. With the mutant *Fl* 39, no photoreaction was observed. These facts had been interpreted previously as indicating that the pool of cytochrome *b*-559, which is photo-oxidizable at 77 K, is different from the pool photo-oxidizable in the presence of ADRY agents at room temperature: the mutant *Fl* 50 lacks the former but has the second pool [11]. Electrophoresis of photosynthetic membranes which have not been heated or lipid depleted separates whole chlorophyll-protein complexes [8]. Figs. 2 and 3 show photographs of gel slabs which were obtained by electrophoresis of PS II-enriched particles isolated from three strains: wild type, *Fl* 39 and *Fl* 50. The results are summarized in

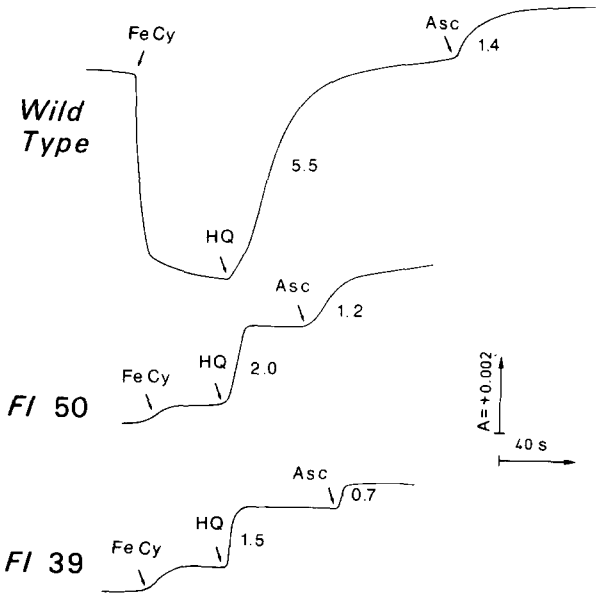


Fig. 1. Kinetics of chemically induced oxidation and reduction of cytochrome *b*-559 in chloroplast fragments of the wild type and of the mutants *Fl* 50 and *Fl* 39 of *C. reinhardii*. Spectrophotometric measurements: the chloroplast fragments were suspended in 0.01 M phosphate buffer (pH 7.5) at concentrations corresponding to 100 $\mu$g Chl $a + b$/ml. Analytical light: 559 nm (reference $\lambda$: 570 nm). Concentrations of the reactants: $2.5 \cdot 10^{-4}$ M $K_3Fe(CN)_6$ (FeCy), $2 \cdot 10^{-3}$ M hydroquinone (HQ) and $4.0 \cdot 10^{-3}$ M sodium ascorbate (Asc). The numbers indicate the total absorbance changes ($\times 10^3$) for 100 $\mu$g Chl $a + b$/ml. Notice that, before $K_3Fe(CN)_6$ addition, the cytochrome was already oxidized in chloroplast fragments of the mutants *Fl* 50 and *Fl* 39 having impaired PS II function, but not in those of the wild type.

Table III. Before staining (Fig. 2), we could observe for the wild type (slots a–d) the five green bands of CP I–CP V (CP V, the least pigmented). For the mutants *Fl* 39 (slots e and f) and *Fl* 50 (slots g and h), CP I and CP II were present but no CP III–CP V bands were observed. After staining with Coomassie brilliant blue (Fig. 3), we could see, for the wild type, bands corresponding to the apoproteins of CP I–CP V. For the same gel, the relative intensities of the CP III–CP V bands appeared greater after staining than before. In particular, the CP V band was often very little pigmented by the chlorophyll, specially in the case of a long time action of LDS before electrophoresis (Fig. 2a and b), but always a well colored protein band corresponded to this fraction. On the other hand, CP III and CP IV did not appear as well separated after staining as before. These facts indicate relatively high protein/chlorophyll ratios for the three

TABLE I

CYTOCHROME *b*-559 CONTENTS OF CHLOROPLAST FRAGMENTS OF THE WILD TYPE AND OF THE MUTANTS *Fl* 9, *Fl* 39, *Fl* 50 OF *C. REINHARDII*

Contents: $\mu$mol chemically oxidized then reduced cytochrome/mmol Chl $a + b$. The measurement of these contents was carried out spectrophotometrically, as shown in Fig. 1.

| Cytochrome *b*-559 | Strains | | | |
|---|---|---|---|---|
| | Wild type | Mutants | | |
| | | *Fl* 9 | *Fl* 39 | *Fl* 50 |
| Hydroquinone reducible | 2.47 | 3.73 | 0.68 | 0.90 |
| Only ascorbate reducible | 0.63 | 0.41 | 0.31 | 0.54 |
| Total ascorbate reducible | 3.10 | 4.14 | 0.99 | 1.44 |

TABLE II

PHOTO-OXIDATION OF CYTOCHROME *b*-559 IN WHOLE CELLS OF THE WILD TYPE AND OF THE MUTANTS *Fl* 9, *Fl* 39, *Fl* 50 OF *C. REINHARDII* IN THE PRESENCE OF THREE ADRY AGENTS

Cytochrome oxidation: $\mu$mol oxidized cytochrome/mmol Chl $a + b$. Spectrophotometric measurements: the cells were suspended in 0.01 M phosphate buffer (pH 7.5) at concentrations corresponding to 100 (wild type, *Fl* 39, *Fl* 50) or 60 (*Fl* 9) $\mu$g Chl $a + b$/ml. Actinic light: 665–720 nm at half-transmission band of the filter (Balzers K7), 25 W/m$^2$. Analytic light: 559 nm (reference $\lambda$: 570 nm). Concentrations of the reactants: $2.4 \cdot 10^{-6}$ M ANT 2p, $2.4 \cdot 10^{-6}$ M ANT 2s, $1.2 \cdot 10^{-5}$ M DCMU, $1.2 \cdot 10^{-4}$ M FCCP.

| Reactants | Strains | | | |
|---|---|---|---|---|
| | Wild type | Mutants | | |
| | | *Fl* 9 | *Fl* 39 | *Fl* 50 |
| FCCP | 1.93 | 2.32 | 0.00 | 0.67 |
| FCCP + DCMU | 0.67 | 0.60 | 0.00 | 0.36 |
| ANT 2p | 1.03 | 1.05 | 0.00 | 0.58 |
| ANT 2p + DCMU | 0.36 | 0.30 | 0.00 | 0.22 |
| ANT 2s | 0.94 | 0.97 | 0.00 | 0.54 |
| ANT 2s + DCMU | 0.31 | 0.37 | 0.00 | 0.27 |

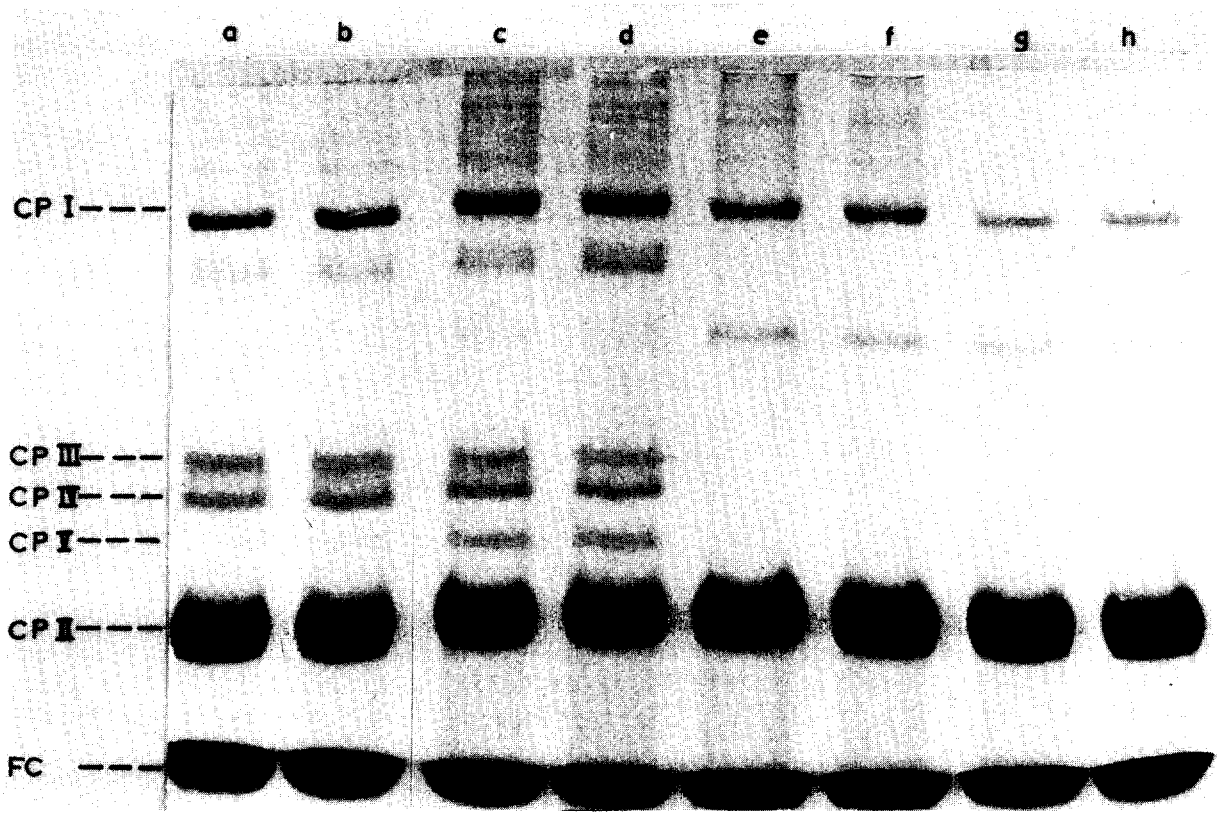


Fig. 2. Chlorophyll-protein complexes of PS II-enriched particles of *C. reinhardii*, wild type (a–d) and mutants *Fl* 39 (e and f) and *Fl* 50 (g and h). The membranes were solubilized in the following mixture: 0.05 M $Na_2CO_3$, 0.05 M dithiothreitol, 10% glycerol, 2% LDS, 0.01% bromophenol blue, to a final chlorophyll concentration of 1 mg/ml and a Chl/LDS ratio of 1 : 20, for 24 h (a and b) or for 15 min (all other cases). They were analyzed by LDS-polyacrylamide gel electrophoresis, at 4°C. Stacking gel: 3% acrylamide and 0.08% *N,N'*-methylbisacrylamide, 0.125 M Tris-HCl buffer (pH 6.8). Resolving gel: 11% acrylamide and 0.20% *N,N'*-methylbisacrylamide, 0.375 M Tris-HCl buffer (pH 8.8). Upper reservoir buffer: 0.025 M Tris, 0.19 M glycine, 0.001 M EDTA and 0.1% LDS (pH 8.4). Lower reservoir buffer: 0.025 M Tris and 0.19 M glycine (pH 8.4). Gel slab thickness: 15 mm, length: 100 mm. Membrane concentration: 30 $\mu$g Chl per slot. Gel before staining: all the bands were green pigmented. The photograph was taken through a blue filter (Wratten 38A). CP, chlorophyll-protein complex; FC, free chlorophyll. Notice that a prolonged action of LDS, at 4°C, before electrophoresis ( a and b) leads to a better separation of CP III and CP IV but also to a decoloration of CP V, as compared to a short time action (c and d).

latter complexes. For the mutants, the protein bands related to CP I and CP II were seen, but none (*Fl* 39) or only traces (*Fl* 50) of those related to CP III–CP V were observable. In addition, for both mutants, a protein band was clearly missing in the lower part of the gel, below CP II. The apparent molecular weight of this protein was approx. 19 000. In fact, traces of this protein appeared for *Fl* 50, but they are not visible on the picture.

In an attempt to localize cytochromes or cytochrome subunits on the electrophoretograms, we used the TMBZ-$H_2O_2$ peroxidation reaction which gives a blue color with the $Fe^{3+}$ of hemes [20,21]. And we analyzed also chloroplast preparations of the mutant *Fl* 9 as reference. This mutant *Fl* 9, which has been previously studied, is devoid of cytochromes *b*-563 and *c*-553 but has functional PS II [12,13]. It has a normal content of cytochrome *b*-559 (Table I) and, as in the wild type, it carried out photo-oxidations of cytochrome *b*-559 both at 77 K and, in the presence

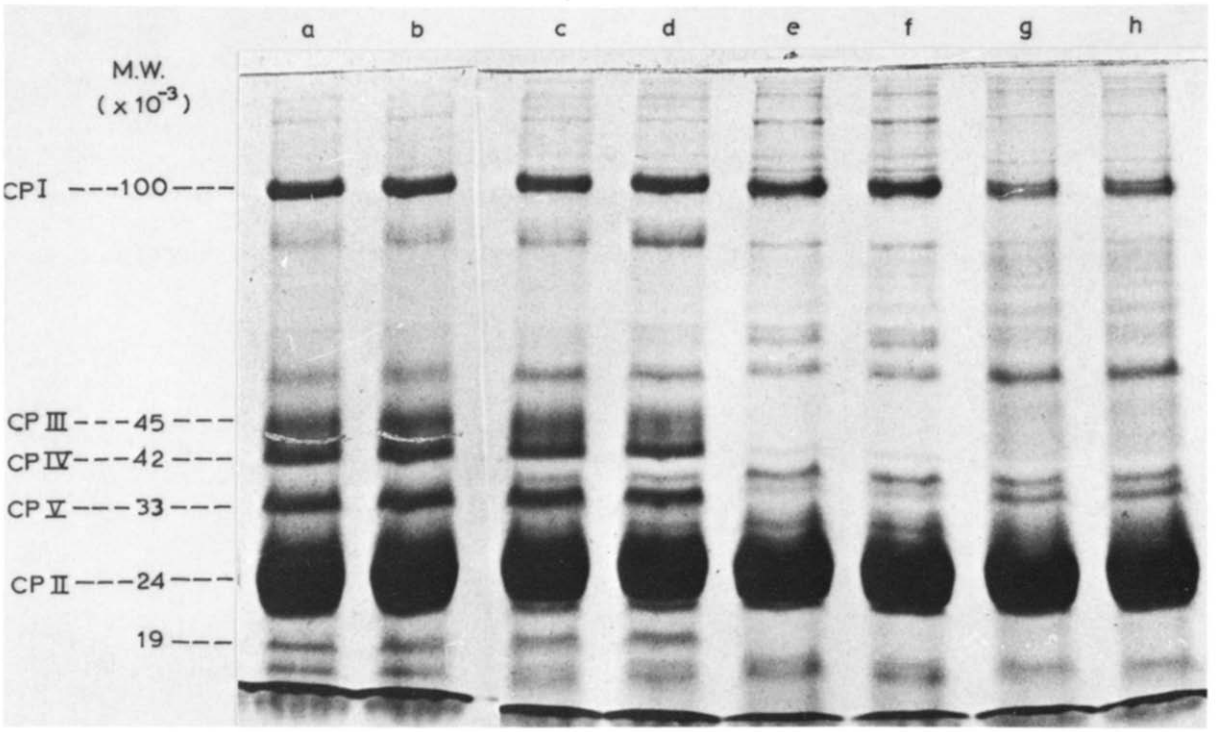


Fig. 3. Proteins of PS II-enriched particles of *C. reinhardii*, wild type (a–d) and mutants *Fl* 39 (e and f) and *Fl* 50 (g and h). Same gel of that of Fig. 2 after staining of the proteins with Coomassie brilliant blue. The photograph was taken through a yellow filter (Minolta Y56). CP, chlorophyll-protein complex; M.W., apparent molecular weights.

TABLE III

OCCURRENCE OF VARIOUS PROTEIN BANDS ON THE ELECTROPHORETOGRAMS OF THE WILD TYPE AND OF THE MUTANTS *Fl* 9, *Fl* 39, *Fl* 50 OF *C. REINHARDII*

Electrophoretograms of PS II-enriched particles or of chloroplasts fragments (see Figs. 2–4). +, band occurring; –, band missing; tr, only traces were visible.

| Bands | Apparent molecular weights | Strains | | | |
|---|---|---|---|---|---|
| | | Wild type | Mutants | | |
| | | | *Fl* 9 | *Fl* 39 | *Fl* 50 |
| Chlorophyll-protein complexes [a] | | | | | |
| CP I | 110 000 | + | + | + | + |
| CP III | 45 000 | + | + | – | tr |
| CP IV | 42 000 | + | + | – | tr |
| CP V | 33 000 | + | + | – | tr |
| CP II | 24 000 | + | + | + | + |
| Heme proteins [b] | 45 000 | + | – | + | + |
| | 42 000 | + | – | + | + |
| | 19 000 | + | – | + | + |
| | 14 000 | + | + | – | – |
| Other protein [c] | 19 000 | + | + | – | tr |

[a] And corresponding apoproteins, stained by Coomassie blue.
[b] Detected by staining with TMBZ and $H_2O_2$.
[c] Visible only after staining with Coomassie blue.

of ADRY agents, at room temperature (Refs. 16 and 22 and Table II). On the electrophoretograms concerning this mutant, the bands corresponding to CP I–CP V, the apoproteins related to these complexes, and the 19 000 dalton protein were clearly observed (not shown). Fig. 4 shows that four different blue bands were distinguished after TMBZ-$H_2O_2$ staining: an intense band in the 40 000–45 000 dalton region which, on the gels, appeared resolved into two separate bands of apparent molecular weights 45 000 and 42 000 (clearly visible only for the mutant *Fl* 39, slots a and b), a 19 000 dalton band and a 14 000 dalton band. Three of these bands (45 000, 42 000 and 19 000 daltons) were shown in all cases except for PS II-enriched particles of the wild type and for chloroplast fragments of the mutant *Fl* 9 (slots e and g). These three bands, which appeared also for the wild type supernatant (slot f), may be attributed to cytochromes *b*-563 and *c*-553. Indeed, both these cytochromes are solubilized by the detergent Triton X-100 [23] and are missing in the mutant *Fl* 9 [12,13]. Though they appeared superposed to CP III and CP IV for the wild type, the 45 000 and 42 000 dalton bands are not part of these complexes; indeed, both these heme proteins were clearly shown for the mutant *Fl* 39 which lacked CP III and CP IV and the related apoproteins. These 45 000 and 42 000 dalton bands were always very close, even in the cases of the mutant *Fl* 39 (slots a and b) and the wild type supernatant (slot f) for

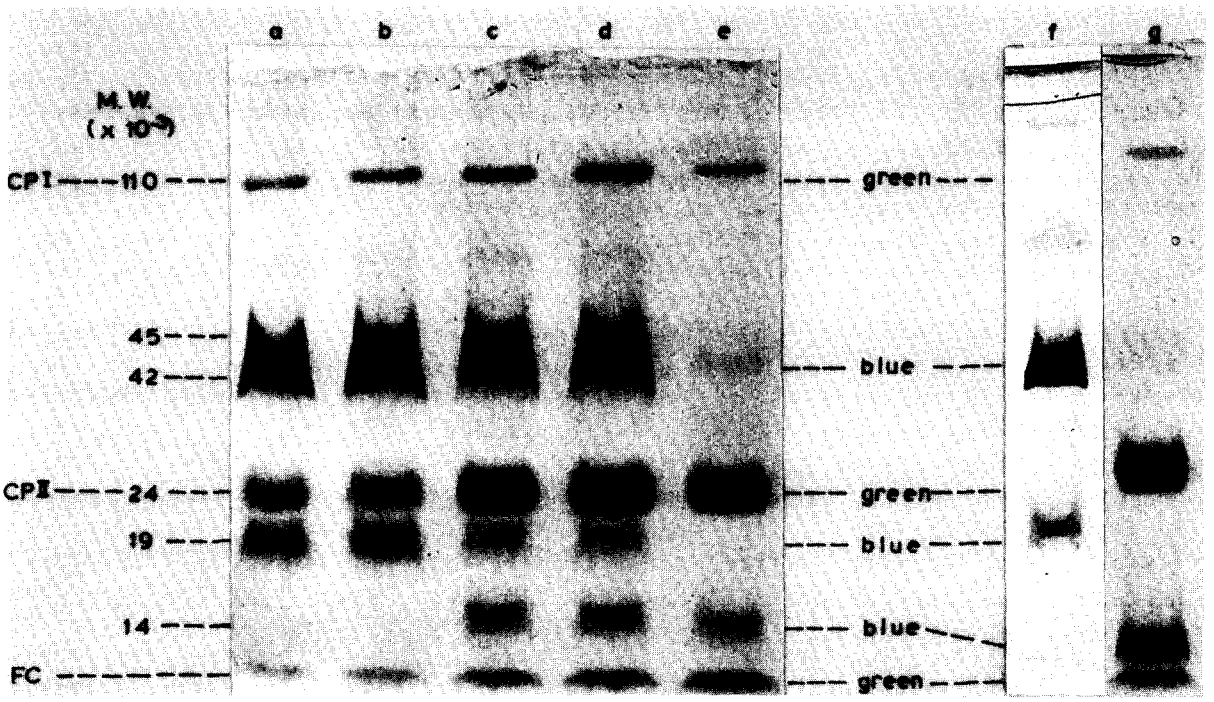


Fig. 4. Heme proteins of chloroplast fragments of *C. reinhardii*, wild type and mutants *Fl* 9 and *Fl* 39. a and b, chloroplast fragments of the mutant *Fl* 39 (cytochrome *b*-559 missing, cytochromes *b*-563 and *c*-553 present); c and d, chloroplast fragments of the wild type (all of the cytochromes present); e and f, Triton X-100-treated chloroplast fragments of the wild type (see Ref. 13): e, pellet (PS II-enriched particles, cytochrome *b*-559 present in a small amount, cytochromes *b*-563 and *c*-553 missing), f, supernatant (cytochrome *b*-559 missing, cytochromes *b*-563 and *c*-553 present); g, chloroplast fragments of the mutant *Fl* 9 (cytochrome *b*-559 present, cytochromes *b*-563 and *c*-553 missing). The membranes were solubilized and analyzed by LDS-polyacrylamide gel electrophoresis, at 4°C, as indicated in the legend of Fig. 2; then the heme proteins having peroxidase activity were stained with TMBZ and $H_2O_2$, according to Refs. 20 and 21. Material concentrations: 30 $\mu$g (*Fl* 39, wild type) or 20 $\mu$g (*Fl* 9) Chl per slot. Green chlorophyll and blue-colored bands appeared together on the gel. The photograph was taken through an orange filter (Minolta 036). CP, chlorophyll-protein complex; FC, free chlorophyll; M.W., apparent molecular weights.

which no excess of chlorophyll-protein complexes could be responsible for a poor separation; this fact suggests that cytochromes *b*-563 and *c*-553 are probably bound. In addition, depending on the gels, either deep 42 000 and clear 19 000 dalton bands or less important 42 000 and deep 19 000 dalton bands were obtained, suggesting that the 19 000 dalton corresponds to a subunit of the 42 000 dalton band. On the other hand, the fourth band, generally less colored, in the lower part of the gels (apparent molecular weight 14 000) was present for the wild type and for the mutant *Fl* 9 (slots c, d and g), but not for the mutant *Fl* 39 and for the wild type supernatant (slots a, b and f). This 14 000 dalton band appeared therefore related to cytochrome *b*-559. The observations which were taken with chloroplast fragments of the mutant *Fl* 50 (not shown on Fig. 4) were similar to those described for *Fl* 39.

It must be noted that the TMBZ-$H_2O_2$ method allows detection of cytochrome quantities in the range of 0.003 nmol per band [20]. This sensitivity is about 15–50-times greater than for the protein staining with Coomassie blue (1–20 $\mu$g per band [24]). Therefore, the heme proteins shown by means of the former method cannot be easily and significantly detected by means of the second.

## Discussion

The mutant *Fl* 39 did not show any PS II activity [10], did not perform any cytochrome *b*-559 photo-oxidation in the presence of FCCP, ANT 2p or ANT 2s, was largely deficient in high-potential cytochrome *b*-559 and did not show CP III–CP V. On the other hand, the mutant *Fl* 50 was not totally devoid of PS II activity: it did not evolve oxygen but its chloroplast fragments were able to carry out very weak photoreduction of 2,6-dichlorophenolindophenol when the electron donor was diphenylcarbazide [10]; it lacked some cytochrome *b*-559 but, in the presence of ADRY agents, it performed clearly cytochrome *b*-559 photo-oxidation, the amplitude of which was 2-times smaller than that of the wild type. In this mutant, traces of CP III–CP V were observed. These results confirm that impaired function of PS II and anomalies concerning cytochrome *b*-559 are correlated. They also corroborate that CP III and CP IV are involved in the photochemistry of PS II [8]; and they provide additional evidence that CP V is also involved in this photochemistry. Finally we found that, for both mutants *Fl* 39 and *Fl* 50, a 19 000 dalton protein was missing. Therefore, it appears that this protein is also related to PS II. This correlation between deficiency in cytochrome *b*-559, impaired PS II function, lack of CP III–CP V, and lack of the 19 000 dalton protein was also observed with another mutant, *Fl* 59, which has properties similar to those of *Fl* 50 [10,11].

Various molecular weights of purified chloroplast cytochromes have been reported in the literature. In the case of the cytochrome *f* of spinach, the molecular weight of the heme-containing chain was evaluated at 31 000 and the total molecular weight at 62 000 [25]. For the algae, the molecular weight of the bound cytochrome *f*-553 of *Scenedesmus acutus* was estimated at 33 000 [26] and that of *Spirulina maxima* at 38 000 [27], but the molecular weight of an *f*-type cytochrome of *Bumilleriopsis filiformis* was only 7 100 [28]. Concerning the cytochrome $b_6$ of spinach, the measured molecular weights were: 18 000 for a simple polypeptide [29]; 60 000 for a lipoprotein, the protein moiety of which had a molecular weight of 40 000 and was composed of 20 000, 9 500 and 6 600 dalton subunits [30]. The molecular weights, which were reported for cytochrome *b*-559, were either 45 900 for a protein composed of eight 5 600 dalton subunits [31] or 37 000 [32] in the case of spinach; it was only 17 000 in the case of the alga *B. filiformis* [32]. Then it appears that, for a given cytochrome, the proposed values depended on the species and also varied somewhat following the experimental conditions used for molecular weight determination (see Ref. 25). Concerning the cytochromes of *C. reinhardii*, no data were found in the literature. Our present results with mutants of this alga do not concern purified cytochromes but are relative to protein fractions separated from whole chloroplast membranes. Nevertheless, they show that three heme proteins of apparent molecular weights 45 000, 42 000 and 19 000 correspond to both cytochromes *b*-563 and *c*-553; but they do not permit us to distinguish between these two cytochromes. In addition, a fourth heme protein, missing in the mutants *Fl* 39 and *Fl* 50, appears related to cytochrome *b*-559; its molecular weight is nearly 14 000. It was reported that sodium dodecyl sulfate has an

inactivating effect on cytochrome *b*-559 [33]. Further studies for separation and localization of the chloroplast cytochromes, by means of less damaging methods, are in progress.

## Acknowledgements

The authors thank Dr. A.-L. Etienne for reading and criticizing this manuscript. The excellent technical assistance of Mrs. J. Charon was greatly appreciated.

## References

1 Thornber, J.P. (1975) Annu. Rev. Plant Physiol. 26, 127–158
2 Anderson, J.M. (1980) Biochim. Biophys. Acta 591, 113–126
3 Chua, N.-H. and Bennoun, P. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 2175–2179
4 Gillham, N.W. (1978) Organelle Heredity, pp. 539–560, Raven Press, New York
5 Hoober, J.K., Millington, R.H. and D'Angelo, L.P. (1980) Arch. Biochem. Biophys. 202, 221–234
6 Metz, J. and Bishop, N.I. (1980) Biochem. Biophys. Res. Commun. 94, 560–566
7 Wild, A. and Urschel, B. (1980) Z. Naturforsch. 35c, 627–637
8 Delepelaire, P. and Chua, N.-H. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 111–115
9 Cramer, W.A. and Whitmarsh, J. (1977) Annu. Rev. Plant Physiol. 28, 133–172
10 Garnier, J., Guyon, D. and Picaud, A. (1979) Plant Cell Physiol. 20, 1013–1027
11 Maroc, J. and Garnier, J. (1979) Plant Cell Physiol. 20, 1029–1040
12 Garnier, J. and Maroc, J. (1970) Biochim. Biophys. Acta 205, 205–219
13 Maroc, J. and Garnier, J. (1973) Biochim. Biophys. Acta 292, 477–490
14 Gorman, D.S. and Levine, R.P. (1965) Proc. Natl. Acad. Sci. U.S.A. 54, 1665–1669
15 Garnier, J. and Maroc, J. (1972) Biochim. Biophys. Acta 283, 100–114
16 Maroc, J. and Garnier, J. (1975) Biochim. Biophys. Acta 387, 52–68
17 Maroc, J. and Garnier, J. (1979) Biochim. Biophys. Acta 548, 374–385
18 Eichenberger, W., Schaffner, J.C. and Boschetti, A. (1977) FEBS Lett. 84, 144–148
19 Lane, L.C. (1978) Anal. Biochem. 86, 655–664
20 Thomas, P.E., Ryan, D. and Levin, W. (1976) Anal. Biochem. 75, 168–176
21 Høyer-Hansen, G. (1980) Carlsberg Res. Commun. 45, 167–176
22 Garnier, J. and Maroc, J. (1975) in Proceedings of the *Third International Congress on Photosynthesis* (Avron, M., ed.), vol. 1, pp. 547–556, Elsevier, Amsterdam
23 Hind, G. and Nakatani, H.Y. (1970) Biochim. Biophys. Acta 216, 223–225
24 Weber, K., Pringle, J.R. and Osborn, M. (1972) Methods Enzymol. 26, 3–27
25 Singh, J. and Wasserman, A.R. (1971) J. Biol. Chem. 246, 3532–3541
26 Böhme, H., Brütsch, S., Weithmann, G. and Böger, P. (1980) Biochim. Biophys. Acta 590, 248–260
27 Ho, K.K. and Krogmann, D.W. (1980) J. Biol. Chem. 255, 3855–3861
28 Lach, H.-J., Ruppel, H.G. and Böger, P. (1973) Z. Pflanzenphysiol. 70, 432–451
29 Lach, H.-J. and Böger, P. (1977) Z. Naturforsch. 32c, 877–879
30 Stuart, A.L. and Wasserman, A.R. (1975) Biochim. Biophys. Acta 376, 561–572
31 Garewal, H.S. and Wasserman, A.R. (1974) Biochemistry 13, 4072–4079
32 Lach, H.-J. and Böger, P. (1977) Z. Naturforsch. 32c, 75–77
33 Garewal, H.S. and Wasserman, A.R. (1974) Biochemistry 13, 4063–4071