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Oligomeric form of the light-harvesting chlorophyll *a* + *b*-protein complex CP II, phosphatidyldiacylglycerol, Δ3-*trans*-hexadecenoic acid and energy transfer in *Chlamydomonas reinhardtii*, wild type and mutants

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Analyses of chlorophyll-protein complexes and of lipids were performed with the wild type of *Chlamydomonas reinhardtii* and three non-photosynthetic mutants: *Fl* 39, which was a 'classical' high-fluorescent Photosystem II (PS II)-lacking mutant, and *mf* 1 and *mf* 2, which lacked also functional PS II but were low-fluorescent and showed an abnormally predominant energy transfer from the main light-harvesting antenna towards Photosystem I. An oligomeric form of the chlorophyll *a* + *b*-protein complex CP II was clearly isolated from the wild type and the mutant *Fl* 39 but it was not detected in the mutants *mf* 1 and *mf* 2. The three mutants showed total lipid contents close to or greater than that of the wild type. Their phosphatidyldiacylglycerol (PG) contents, on a chlorophyll basis, were higher (*Fl* 39) or 1.4- (*mf* 1) and 2.0- (*mf* 2) times lower than that of the wild type. The fatty acid compositions of the wild type and of the mutant *Fl* 39 were comparable, showing about equal amounts of a C18 series and a C16 series which included the Δ3-*trans*-hexadecenoic acid (C16:1-*trans*). This C16:1-*trans* was not detected in the mutants *mf* 1 and *mf* 2 which contained the other fatty acids. These results indicate correlations between lack of C16:1-*trans*-containing PG, lack of an oligomeric form of CP II and an impaired mechanism of the regulation of excitation energy transfer from the main chlorophyll *a* + *b* antenna.

Abbreviations: C16:0, palmitic acid; C16:1Δ7, Δ7-*cis*-hexadecenoic acid; C16:1Δ9, Δ9-*cis*-hexadecenoic acid; C16:1-*trans*, Δ3-*trans*-hexadecenoic acid; C16:2, Δ7,10-*cis*,*cis*-hexadecadienoic acid; C16:3, Δ7,10,13-all *cis*-hexadecatrienoic acid; C16:4, Δ4,7,10,13-all *cis*-hexadecatetraenoic acid; C18:0, stearic acid; C18:1Δ7, Δ7-*cis*-octadecenoic acid; C18:1Δ9, Δ9-*cis*-octadecenoic acid; C18:2, Δ9,12-*cis*,*cis*-octadecadienoic acid; C18:3Δ6, Δ6,9,12-all *cis*-octadecatrienoic acid; C18:3Δ9, Δ9,12,15-all *cis*-octadecatrienoic acid; Chl, chlorophyll; DCIP, 2,6-dichlorophenolindophenol; $DCIPH_2$, reduced 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHCP, light-harvesting chlorophyll *a* + *b*-protein complex; PG, phosphatidyldiacylglycerol; Pipes, 1,4-piperazinediethanesulfonic acid; PS I, Photosystem I; PS II, Photosystem II.

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Introduction

In higher plants and green algae, the main light-harvesting antenna for the two photosystems is a chlorophyll (Chl) *a* + *b*-protein complex, commonly designated LHCP or CP II, which contains about half the total Chl of the chloroplast (see reviews in Refs. 1 and 2). Monomeric and oligomeric forms of higher plant LHCP have been frequently observed, the higher oligomeric form ($LHCP^1$) being often more abundant and resembling more closely the in vivo state than the monomeric form ($LHCP^3$) [3–6]. Concerning green algae, oligomeric forms of LHCP have been rarely

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observed. However, such oligomeric forms have been isolated from *Chlamydomonas reinhardtii* [7] and from *Chlorella fusca* [8] but they appeared less abundant than the monomeric forms on the electrophoretograms.

Lipids are associated with the Chl-protein complexes in the thylakoid membrane. For higher plants, the main lipids are glycolipids: monogalactosyldiacylglycerol and digalactosyldiacylglycerol, rich in tri-unsaturated fatty acids, which represent about 80% of the chloroplast lipids and sulfoquinovosyldiacylglycerol which represents about 10%. Besides these lipids, a phospholipid, phosphatidyldiacylglycerol (PG), is also present which represents about 8% of the chloroplast lipids and which contains an unusual fatty acid specific to chloroplasts, the Δ3-*trans*-hexadecenoic acid (C16 : 1-*trans*) [9,10]. Several kinds of evidence, reported by various authors, have indicated that C16 : 1-*trans*-containing PG could be involved in the formation and the stability of an oligomeric form of LHCP and, consequently, could act on the efficiency of the light energy capture and transfer (see Refs. 11–13 and, for a review, Ref. 14). However, recent observations with a mutant of *Arabidopsis thaliana*, which lacked C16 : 1-*trans* but showed LHCP oligomeric form, do not agree with this implication of C16 : 1-*trans* in the formation of oligomeric forms of LHCP [15,16]. Concerning the lipids of the green alga *C. reinhardtii*, analyses have pointed out the presence of the usual glycolipids mono- and digalactosyldiacylglycerol and sulfoquinovosyldiacylglycerol and of phospholipids as phosphatidylethanolamine, phosphatidylinositol and PG, while only traces of phosphatidylcholine were detected; in addition an unusual lipid, trimethylhomoserine, was also found. These lipids contained a large set of C16 and C18 fatty acids, including the characteristic C16 : 1-*trans* which was specifically found in PG [17–19]. Thus all the typical lipids found in higher plant chloroplasts are present in *C. reinhardtii*.

The present paper reports results of analyses of the chlorophyll-protein complexes and of the lipids of the wild type of *C. reinhardtii* and of three non-photosynthetic mutants previously isolated in our laboratory. Two of these mutants were low-fluorescent and showed anomalies which affect the regulation of the distribution of the light energy captured by the main light-harvesting antenna towards the photochemical centers. The third mutant, used as a control, was high fluorescent. It appears that both the low-fluorescent mutants lacked an oligomeric form of the light-harvesting Chl *a* + *b*-protein complex CP II and the fatty acid C16 : 1-*trans*, suggesting a correlation between both these deficiencies and an impaired excitation energy transfer.

Materials and Methods

The characteristics of the wild type of *C. reinhardtii* and of the high-fluorescent mutant *Fl* 39 have been indicated in preceding papers [20–23]. The low-fluorescent mutants *mf* 1 and *mf* 2 have been more recently isolated and described [24].

Algae were grown in light, in Tris-acetate-medium [25] as previously reported [26]. The Chl contents were measured according to refs. 27 and 28. The photoreduction of DCIP and the oxygen uptake by cells disrupted by mild sonication [20,23], the fluorescence induction kinetics of whole cells [22] and the low-temperature fluorescence emission spectra [23] were measured as previously described.

The Triton X-100-treated chloroplast particles from *C. reinhardtii* were prepared as previously indicated [23]. The chloroplasts from lettuce were prepared according to Ref. 29. The Chl-protein complexes were analyzed, without lipid extraction or heating, by lithium dodecylsulfate-polyacrylamide gel electrophoresis at 4 °C, according to Refs. 21, 22 and 30 but using a solubilization mixture containing 0.88% *n*-octyl-β-D-glucopyranoside and 0.22% sodium dodecylsulfate [23,31] to which 50 mM dithiothreitol was added. For polypeptide analysis, gel fragments containing isolated Chl-protein complexes were soaked in 4% lithium dodecylsulfate at room temperature. The polypeptides from these soaked gel fragments and from Triton X-100-treated chloroplast particles were analyzed by lithium dodecylsuflate-polyacrylamide gel electrophoresis, at 4 °C, using a 12–20% polyacrylamide gradient gel, as previously indicated [22].

For lipid analysis, a pellet of algae corresponding to $(4–8) \cdot 10^8$ cells (18–36 mg dry matter) was fixed in boiling ethanol for 5 min, then the lipids

were extracted in chloroform according to Ref. 32. An aliquot of the total lipid extract was transmethylated for fatty acid analysis and another aliquot was used for lipid classes separation by thin-layer chromatography, as previously described [33]. The different spots were visualized under ultraviolet light after primuline pulverization; the spot corresponding to PG was scraped off and transmethylated for fatty acid analysis. The fatty acid methylesters were analyzed by capillary gas-liquid chromatography, using a Girdel 30 chromatograph equipped with a flame ionization detector. A 25 m length, 0.32 mm diameter Carbowax glass column was used, isothermally at 170 °C.

Results

Chlorophylls, photochemical activities and fluorescence

Detailed studies of the pigments and the functional properties of the wild type and the mutant *Fl* 39 of *C. reinhardtii* [20–23] and of the mutants *mf* 1 and *mf* 2 [24] have already been reported elsewhere. Therefore, only some necessary characteristic data are summarized in Table I.

Like the wild type, the three mutants contained Chl *a* and Chl *b* and showed Chl *a*/Chl *b* ratios higher than 2. Their total Chl contents were 1.3 (*Fl* 39), 2.1 (*mf* 1) and 1.5 (*mf* 2) times lower than that of the wild type. The three mutants did not carry out the Hill reaction $H_2O \rightarrow$ DCIP, indicating non-functional Photosystem II (PS II) in these strains. On the other hand, the three mutants clearly carried out the Mehler reaction $DCIPH_2 \rightarrow$ methyl viologen, indicating functional Photosystem I (PS I).

Like numerous PS II-lacking mutants of algae and higher plants [34], the mutant *Fl* 39 showed minimum and steady-state fluorescence yields clearly higher than those of the wild type, whereas the maximum yields of both these strains were relatively close. On the other hand, the mutants *mf* 1 and *mf* 2 showed maximum fluorescence yields (F_{max}) respectively 4- and 3-times lower than that of the wild type, whereas the minimum yields (F_0) and the steady state yields (F_{st}) of the three strains were not very different.

Low-temperature fluorescence emission spectra of cells of the wild type and of the mutants *Fl* 39, *mf* 1 and *mf* 2 are shown in Fig. 1. The spectrum of the wild type indicated a fluorescence of PS II (emissions at 686 and 696 nm) more important than that of PS I (peak around 714 nm) and the spectrum of the PS II-lacking mutant *Fl* 39 showed, besides the PS I emission around 714 nm, a great emission of the light-harvesting antenna CP II at 680–682 nm [23]. But, on the spectra of the mutants *mf* 1 and *mf* 2, the contribution of the fluorescence of CP II was reduced to a weak shoulder around 682 nm and the contribution of the fluorescence of PS I in the 714 nm region appeared clearly preponderant. We have observed elsewhere that, in both these mutants, the excitation energy from the light-harvesting antenna CP II was always mainly transferred towards PS I

TABLE I

CHLOROPHYLL CONTENTS, PHOTOCHEMICAL ACTIVITIES AND CHARACTERISTICS OF THE CHLOROPHYLL FLUORESCENCE YIELDS OF THE WILD TYPE AND OF THE MUTANTS *Fl* 39, *mf* 1 AND *mf* 2 OF *C. REINHARDTII*

(a) and (b) μg chlorophyll per mg dry matter; (c) μmol of reduced DCIP per min per mg of Chl *a* + *b* (spectrophotometric measurement of the photoreduction of DCIP); (d) μmol of absorbed oxygen per min per mg of Chl *a* + *b* (amperometric measurement of oxygen photo-absorption in the presence of methyl viologen); (e), (f) and (g) ratios of fluorescence yields of whole cells suspended in phosphate buffer (pH 7.5), at 20 °C, at a concentration corresponding to 30 μg of Chl *a* + *b* per ml. F_0, initial minimum fluorescence yield at the onset of illumination; F_{max}, maximum fluorescence yield measured in the presence of 10 μM DCMU; F_{st}, steady state fluorescence yield measured 2 min after the start of illumination.

Measurements	Wild type	Mutants		
		Fl 39	*mf* 1	*mf* 2
Chlorophyll contents				
(a) Chl *a*	34.5	27.8	17.1	23.1
(b) Chl *b*	15.1	11.3	6.5	10.4
Photochemical activities				
(c) $H_2O \rightarrow$ DCIP	1.28	0.00	0.00	0.00
(d) $DCIPH_2 \rightarrow$ methyl viologen	3.92	2.17	5.98	4.30
Ratios of fluorescence yields				
(e) F_0 mutant/F_0 wild type	–	3.34	0.86	1.38
(f) F_{max} mutant/F_{max} wild type	–	0.85	0.25	0.33
(g) F_{st} mutant/F_{st} wild type	–	1.92	0.58	0.82

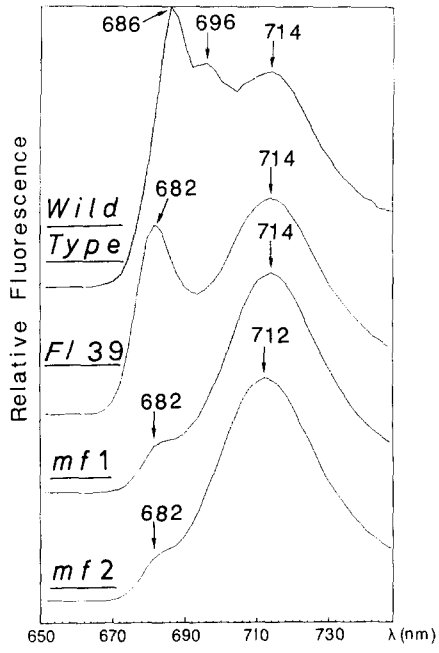

Fig. 1. Low-temperature fluorescence emission spectra of cells of the wild type and of the mutants *Fl* 39, *mf* 1 and *mf* 2 of *C. reinhardtii*. The cells (50 μg of Chl $a + b$ per ml) were suspended in phosphate buffer (pH 7.5), then frozen in 0.1 mm thickness cuvettes which were set against the front of the optical guide of the spectrofluorimeter and plunged into liquid nitrogen. Excitation light wavelength, 450 nm. Slit of the analytical monochromator, 2 nm. The spectra were normalized at their maxima at 712 or 714 nm. The numbers indicate the wavelengths of the peaks and shoulders.

whatever the oxido-reduction level of the plastoquinone pool [24]. All these particularities may be interpretated as indicating an impaired regulation mechanism for the distribution of the excitation energy from the main light-harvesting antenna, leading to an abnormally preferential transfer towards PS I.

Chlorophyll-protein complexes

Fig. 2 shows an electrophoretogram of the Chl-protein complexes of Triton X-100-treated chloroplast particles of the wild type and of the mutants *Fl* 39, *mf* 1 and *mf* 2 of *C. reinhardtii* and, in addition, of chloroplasts of lettuce. The following bands were shown on the electrophoretogram of the wild type (lane a): CP Ia, which corresponds to the undissociated PS I complex (see Ref. 35), and four bands already observed previously by us [21–23]: CP I which corresponds to the core antenna and the reaction center of PS I; CP III and CP IV which were not separated here and which correspond to the reaction center of PS II and its antennae; CP II which corresponds to the main Chl $a + b$ light-harvesting antenna for both photosystems; CP V, a Chl $a + b$ complex related to the PS II antenna which appeared as a shadowy band just above CP II. In addition, a sixth band with an apparent M_r in the

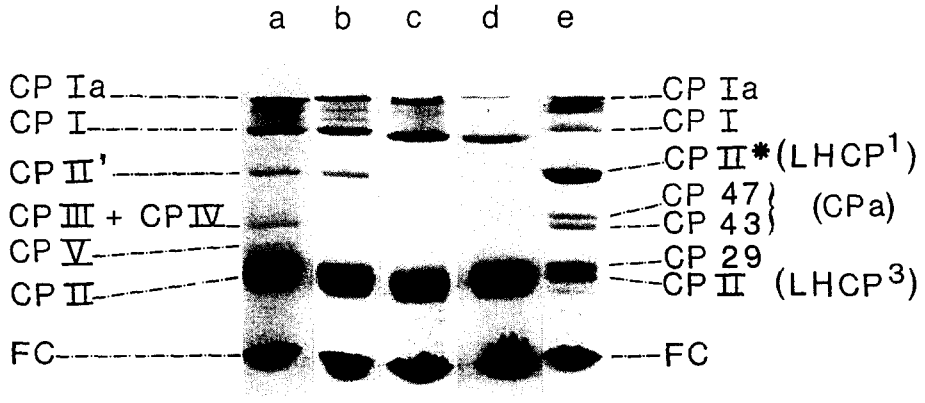

Fig. 2. Chlorophyll-protein complexes of the wild type (a) and the mutants *Fl* 39 (b), *mf* 1 (c) and *mf* 2 (d) of *C. reinhardtii* and of lettuce (e). (a–d) Triton X-100-treated chloroplast particles (25 μg of Chl $a + b$ per well); (e) chloroplasts (22 μg of Chl $a + b$ per well). The membranes were solubilized in 0.88% *n*-octyl-β-D-glucopyranoside, 0.22% sodium dodecylsulfate, 50 mM dithiothreitol, 20 mM Pipes buffer (pH 6.6), 15 mM NaCl, 5 mM $MgCl_2$ and 100 mM sucrose. Lithium dodecylsulfate-polyacrylamide gel electrophoresis was then performed at 4° C, using an 11% polyacrylamide gel, as indicated in Refs. 21 and 22. Unstained gel: all the bands were green pigmented. The photograph was taken through a blue filter (Wratten 38A). In the left, *C. reinhardtii* nomenclature of Chl-protein complexes according to Refs. 30 and 35; in the right, higher plant nomenclature according to Ref. 31 and, in parentheses, according to Refs. 4 and 36. FC, free chlorophyll.

68 000 range was present between CP I and CP III + CP IV. This band, designated CP II′, had not been observed in *C. reinhardtii* up until now, except by Ladygin et al. [7] who have described a Chl *a* + *b*-protein complex LH1 which may correspond to our present CP II′. These authors have suggested that this LH1 complex was an oligomer of another, more abundant, LH3 complex which seems to correspond to our CP II, but they did not perform any polypeptide analysis. This electrophoretogram of the wild type was very similar to that of lettuce chloroplasts (lane e) which showed the following bands, according to Dunahay et al. [31]: CP Ia, CP I, CP II*, CP 47, CP 43, CP 29 and CP II. CP Ia, CP I and CP II designated similar complexes as in *C. reinhardtii*, CP 47 corresponds to CP III, CP 43 to CP IV, CP 29 to CP V and CP II* to the present CP II′. According to several authors [4,13,36], the band CP II* (also designated $LHCP^1$) corresponds to an oligomeric form of the light-harvesting Chl *a* + *b*-complex CP II (also designated $LHCP^3$). On the electrophoretograms of the mutants *Fl* 39 (lane b), *mf* 1 (lane c) and *mf* 2 (lane d), CP Ia, CP I and CP II were present but CP III, CP IV and CP V were absent, confirming the absence of PS II in these strains (the weakness of the CP Ia band in *mf* 2 was an electrophoretic accident: indeed, other analyses have indicated normal amounts of CP Ia in this mutant). In addition, a band of CP II′ was clearly seen for *Fl* 39, but no band corresponding to this complex was observed for *mf* 1 and *mf* 2.

Fig. 3 lanes a, b and c show electrophoretograms of the proteins from Triton X-100-treated chloroplast particles of the wild type and of the mutants *mf* 1 and *mf* 2, separated on a 12–20% polyacrylamide gradient gel. It appears clearly that both the mutants lacked the PS II-related polypeptides of $M_r = 50\,000$, 47 000 and 19 000 and had polypeptides of $M_r = 33\,000$ in lower amounts than that of the wild type, confirming the absence of functional PS II in these mutants (see Ref. 22). Whether the mutants *mf* 1 and *mf* 2 lacked or did not lack the fifth PS II-related polypeptide, of $M_r = 27\,000$, was not easily discernible because of the presence of polypeptides from CP II in this region. Lanes d and e correspond to a second electrophoresis of isolated CP II and CP II′. Five polypeptide bands were observed for CP II′ (lane d): four clearly visible and a pale fifth (just under the upper band) more difficult to be discerned on the photograph. These polypeptides had apparent M_r between 24 000 and 27 000, as evaluated from known standard proteins. They corresponded exactly to the five

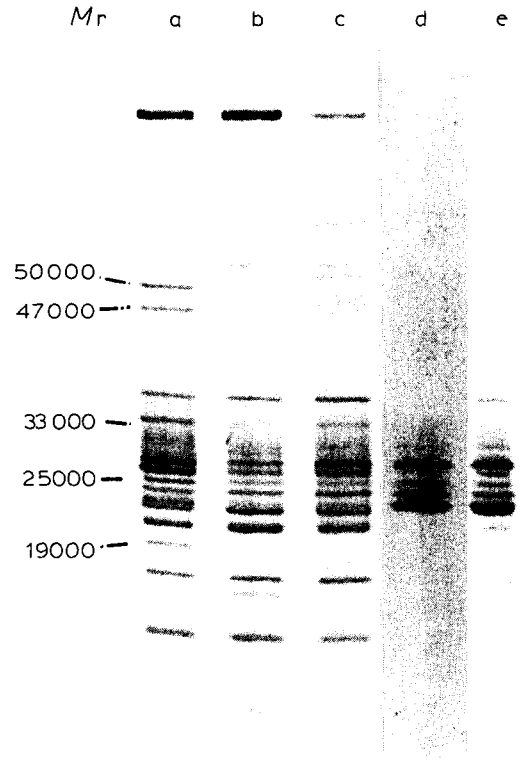

Fig. 3. Polypeptides of Triton X-100-treated chloroplast particles (a–c) and of isolated chlorophyll-protein complexes CP II′ and CP II (d, e) of *C. reinhardtii*. (a–c) Triton X-100-treated chloroplast particles of the wild type (a) and the mutants *mf* 1 (b) and *mf* 2 (c) were solubilized at 4° C in 0.05 M Na_2CO_3, 0.05 M dithiothreitol, 10% glycerol and 2% lithium dodecylsulfate and the solutions were set on the gel at concentrations corresponding to 10 μg of Chl *a* + *b* per well. (d, e) Gel fragments containing CP II′ (d) or CP II (e) from the mutant *Fl* 39, which had been first isolated in non-denaturing conditions as indicated in Fig. 2, were excised and soaked for 90 min, at room temperature, in 0.05 M Na_2CO_3, 0.05 M dithiothreitol, 10% glycerol and 4% lithium dodecylsulfate; then they were set directly in wells of the gel, the protein quantities corresponding to 52 μg (d) and 11 μg (e) of Chl *a* + *b* on the first preparative gel. Lithium dodecylsulfate-polyacrylamide gel electrophoresis was performed at 4° C, using a 12–20% polyacrylamide gradient gel, as indicated in Ref. 22. The polypeptides were stained with Coomassie brilliant blue. The photograph was taken through a yellow filter (Minolta Y52).

main bands which were observed for CP II (lane e). The other bands appearing on the CP II electrophoretogram were probably contaminations by polypeptides from CP Ia, which had migrated in the same region as CP II during the first electrophoresis. These five polypeptides from CP II and CP II′ corresponded probably to the polypeptides nrs. 11, 14, 15, 16 and 17, with apparent M_r between 25 000 and 30 500, which have been observed by Delepelaire and Chua [30] for heated purified CP II of *C. reinhardtii*.

On the other hand, it was observed that low-temperature emission spectra of isolated complexes showed a similar unique peak at 680–681 nm for CP II′ as for CP II. In addition, we searched for CP II′ in six other photosynthesis mutants having various defects: it was detected in all strains except in one which was devoid of Chl *b* and of CP II. All these observations are in agreement; they confirm that CP II′ corresponds to an oligomeric form of CP II.

Lipids

Table II indicates the total lipid and PG contents of cells of the wild type and of the mutants *Fl* 39, *mf* 1 and *mf* 2 of *C. reinhardtii*. The three mutants showed contents on a dry matter weight basis relatively close to that of the wild type. On a Chl basis, the lipid contents of the mutants appeared clearly higher than that of the wild type. On the other hand, the PG contents of the different strains were not similar. The mutant *Fl* 39 showed PG contents greater than that of the wild type on both dry matter weight and Chl bases. But, if the mutants *mf* 1 and *mf* 2 appeared markedly depleted in PG on a dry matter weight basis, their PG contents were respectively only 1.4- and 2.0-times lower than that of the wild type on a Chl basis. Since PG is mainly located in chloroplasts, this latter basis seems more appropriate. Thus, even in the *mf* 2 case, the chloroplasts of these mutants did not appear to be very deficient in PG.

The compositions in the different fatty acids of cells of the four strains are indicated in Table III. The composition of the wild type was comparable to that previously published by Eichenberger [18]. It showed two fatty acid series in about equal amounts: a C16 series and a C18 series. Within the C16 series, the acid C16 : 1-*trans*, characteristic of the thylakoid membranes, was present. This C16 : 1-*trans* was also present in the mutant *Fl* 39, but it was not detected in the mutants *mf* 1 and

TABLE II

TOTAL LIPID AND PHOSPHATIDYLDIACYLGLYCEROL CONTENTS OF THE CELLS OF THE WILD TYPE AND OF THE MUTANTS *Fl* 39, *mf* 1 AND *mf* 2 OF *C. REINHARDTII*

(a) Contents in mg of fatty acids per g dry matter; (b) contents in μg of fatty acid per mg of Chl $a + b$. The fatty acids of total lipid extracts or of isolated phosphatidyldiacylglycerol were transmethylated then analyzed by capillary gas-liquid chromatography. The lipid classes were separated by thin-layer chromatography (see Materials and Methods).

Lipids	Wild type	Mutants		
		Fl 39	*mf* 1	*mf* 2
Total lipids				
(a)	32.3	39.0	36.8	39.9
(b)	651	998	1305	1191
Phosphatidyldiacylglycerol				
(a)	1.9	2.6	0.6	0.6
(b)	37.8	66.7	26.3	18.8

TABLE III

FATTY ACID COMPOSITIONS OF THE CELLS OF THE WILD TYPE AND OF THE MUTANTS *Fl* 39, *mf* 1 AND *mf* 2 OF *C. REINHARDTII*

Compositions in percentage of total fatty acid contents. The fatty acids of total lipid extracts were transmethylated then analyzed by capillary gas-liquid chromatography, as indicated in the Materials and Methods.

Fatty acids	Wild type	Mutants		
		Fl 39	*mf* 1	*mf* 2
C16 : 0	15.3	19.9	17.5	17.5
C16 : 1-Δ7 [a]	5.6	6.8	6.4	3.3
C16 : 1-Δ9 [a]	4.6	1.6	2.3	9.6
C16 : 1-*trans*	2.3	1.5	0.0	0.0
C16 : 2	0.8	2.7	1.4	0.9
C16 : 3	0.8	1.2	2.3	0.7
C16 : 4	18.0	18.9	19.5	13.7
C18 : 0	2.4	2.3	1.9	1.7
C18 : 1-Δ7 [a]	10.2	6.2	2.1	19.9
C18 : 1-Δ9 [a]	4.3	4.9	6.0	4.4
C18 : 2	5.4	12.4	6.2	4.7
C18 : 3-Δ6	7.1	7.4	10.5	7.4
C18 : 3-Δ9	23.2	14.2	23.9	16.2

[a] Tentatively identified as Δ7 or Δ9.

mf 2. Except for this noteworthy deficiency of *mf* 1 and *mf* 2 in C16 : 1-*trans*, the fatty acid compositions of the three mutants were not very different of that of the wild type, the acids C16 : 0, C16 : 4 and C18 : 3Δ9 being the most abundant in the four strains. Nevertheless, the C18 : 2 content of *Fl* 39 and the C16 : 1Δ9 and C18 : 1Δ7 contents of *mf* 2 appeared to be relatively greater than the corresponding contents of the other strains.

The absence of C16 : 1-*trans* in the mutants *mf* 1 and *mf* 2 was confirmed by analysis of the fatty acids from isolated PG. Fig. 4 illustrates the fatty acid pattern in PG fractions isolated from the four strains. It appears clearly that C16 : 0 and C16 : 1-*trans* were the major fatty acids in the PG of the wild type and the mutant *Fl* 39. In the PG of the mutants *mf* 1 and *mf* 2, C16 : 0 was also the major fatty acid but no C16 : 1-*trans* was observed. The same five C18 acids appeared in the PG of the four strains. Their relative quantitative variations do not seem to be meaningful: indeed, different analyses of the PG of a same strain showed comparable or greater variations. On the other hand, fatty acid analyses for all the other lipid classes showed that: (1) in the wild type and in the mutant *Fl* 39, C16 : 1-*trans* was exclusively found in PG and (2) in the mutants *mf* 1 and *mf* 2, this fatty acid was not detected in any other lipid class.

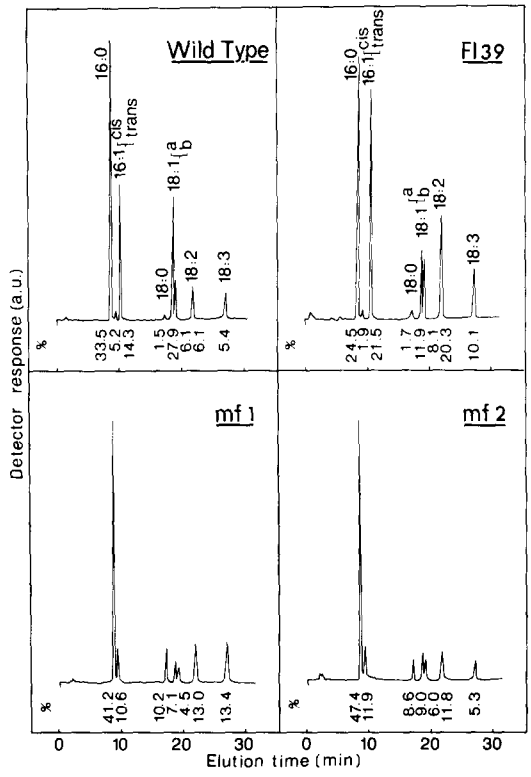

Fig. 4. Fatty acid compositions of phosphatidyldiacylglycerol (PG) from the wild type and the mutants *Fl* 39, *mf* 1 and *mf* 2 of *C. reinhardtii*. The fatty acid from PG, which had been isolated by thin-layer chromatography starting from 50 mg of algal dry matter, were transmethylated then analyzed by capillary gas liquid chromatography, as indicated in Materials and Methods. The percentages indicate the relative importance of the areas of the different peaks. (a) Tentatively identified as 18 : 1-Δ7; (b) Tentatively identified as 18 : 1-Δ9; a.u., arbitrary unit.

Discussion

The use of *n*-octyl-β-D-glucopyranoside + dithiothreitol in the solubilization mixtures allowed us to separate clearly from chloroplast particles of *C. reinhardtii* a chlorophyll-protein complex CP II′ which appears to be an oligomeric form of the light-harvesting Chl $a + b$-protein complex CP II and to correspond to the CP II* (LHCP[1]) of higher plants. This complex CP II′ was observed in the wild type and in the high-fluorescent mutant *Fl* 39 of *C. reinhardtii*, but it was absent in the low-fluorescent mutants *mf* 1 and *mf* 2 in which the excitation energy from the light-harvesting Chl $a + b$ antenna CP II was abnormally transferred preferentially towards PS I. Thus, in the case of these two latter mutants, a correlation appears between the lack of the oligomeric form of the main light-harvesting complex and an impaired mechanism of the light energy distribution. It is possible that, as in higher plants [1,4,37], this oligomeric form of CP II corresponds to an in vivo form of the main antenna in *C. reinhardtii* and plays an essential role in the regulation of the excitation energy distribution between the two photosystems.

Numerous works by different authors have suggested that, in higher plants, C16 : 1-*trans*-containing PG is directly implicated in the oligomeric structure of the light-harvesting Chl-protein complex and, consequently, in the efficiency of light collection [12–14,36,38]. On the other hand,

Browse et al. [15] and McCourt et al. [16] showed that the oligomeric forms of LHCP and of PS I Chl-protein complex appeared more labile to detergent- or cation-mediated dissociation in a mutant of *Arabidopsis thaliana* which lacked C16 : 1-*trans* than in the corresponding wild type, but they observed no significant modification of the efficiency of energy transfer from LHCP to the photochemical reaction centers nor any effect on the thermal stability of the Chl-protein complexes in vivo. More recently, Huner et al. [39], Krol et al. [40] and Williams et al. [41] have observed a correlation between a decrease in C16 : 1-*trans* associated with PG and a decrease in oligomerization of the light-harvesting complex LHCP II in *Secale cereale* which was exposed to a low temperature during greening and growth. Thus, if there are relations between C16 : 1-*trans* and the organization of the light-harvesting antenna, the precise function of this fatty acid is not yet completely elucidated. Our present findings show that the mutants *mf* 1 and *mf* 2 of *C. reinhardtii*, which showed anomalies concerning the regulation of the excitation energy distribution from the main Chl $a + b$ antenna, lacked C16 : 1-*trans* and CP II′, the oligomeric form of CP II. In addition, the fact that the mutant *mf* 1 showed a PG content close to that of the wild type on a Chl basis and the occurrence of in vivo impaired energy transfer in both these mutants rule out the possibility that the absence of CP II′ in these strains was only due either to an unspecific deficiency in total PG or to a simple increase of the lability of the oligomeric form of CP II to detergent-mediated dissociation.

In conclusion, these observations with mutants of a green alga are in favour of a strong correlation between the presence of C16 : 1-*trans*-containing PG, the formation and stability of an oligomeric form of the Chl $a + b$-protein complex CP II and an efficient regulation of the energy transfer from the main light-harvesting antenna towards the photochemical centers. But the precise function of C16 : 1-*trans*-containing PG and also the possibility that another factor could be implicated in the formation of oligomeric forms of CP II are not yet completely elucidated. Experiments of supplementation with exogenous fatty acids or of genetic complementation, made with the mutants *mf* 1 and *mf* 2, would be probably of interest to answer these questions.

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