Biochimica et Biophysica Acta, 1020 (1990) 153–162
Elsevier

BBABIO 43288

Restoration of both an oligomeric form of the light-harvesting antenna CP II and a fluorescence state II-state I transition by Δ^3-*trans*-hexadecenoic acid-containing phosphatidylglycerol, in cells of a mutant of *Chlamydomonas reinhardtii*

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(Received 29 March 1990)
(Revised manuscript received 10 July 1990)

Key words: Δ^3-*trans*-Hexadecenoic acid; Excitation energy transfer; Light-harvesting chlorophyll-protein complex; Lipid; Photosynthesis; (*C. reinhardtii*)

To define the role of the Δ^3-*trans*-hexadecenoic acid (16:1-*trans*) in the thylakoid membrane, restoration experiments were carried out using a Photosystem II (PS II)-lacking but low-fluorescent mutant, *mf* 2, of *Chlamydomonas reinhardtii*. This mutant is unable to synthesize 16:1-*trans*, lacks an oligomeric form of the main light-harvesting chlorophyll (Chl) *a* + *b* antenna, CP II, and shows an impaired regulation of the excitation energy distribution. Whole cells were incubated for 39 h in the presence of liposomes of 16:1-*trans*-containing phosphatidylglycerol (PG-16:1-*trans*), in the light at 25 °C. Then lipids and Chl-protein complexes were analyzed and low-temperature fluorescence emission spectra, both in state I and in state II (oxidized and reduced plastoquinone pool), were measured. The results indicated: (1) a relatively important content of 16:1-*trans* specifically incorporated in the chloroplast phosphatidylglycerol (PG); (2) an appreciable amount of CP II oligomeric form; (3) the occurrence of a clear state II-state I transition, as shown by a ratio of the CP II fluorescence at 682 nm to the photosystem I fluorescence at 712 nm, which was 3.8-times higher in state I than in state II. These restorations were not observed when cells of *mf* 2 were incubated in the presence of palmitate-containing PG, of oleate-containing phosphatidylcholine or of PG-16:1-*trans* + cycloheximide. It is concluded that: (1) the oligomeric form of CP II is essential for a good excitation energy transfer towards the PS II region and, consequently, to a good state II-state I transition in the distribution of excitation energy; (2) PG-16:1-*trans* probably plays an essential role in stabilizing neo-formed CP II oligomers during the assembly of new Chl-protein complexes in the chloroplast.

Introduction

In thylakoids of higher plants, about 70% of the phosphatidylglycerol (PG) and the totality of the Δ^3-*trans*-hexadecenoic acid (16:1-*trans*), a specific fatty acid of chloroplasts, are located in the outer layer of the membrane, all the 16:1-*trans* being esterified in PG [1,2]. Several roles have been proposed for this 16:1-*trans*-containing PG (PG-16:1-*trans*) [1,3,4]. In particu-

Abbreviations: 16:0, hexadecanoic acid (palmitic acid); 16:1Δ9, Δ^9-*cis*-hexadecenoic acid; 16:1-*trans*, Δ^3-*trans*-hexadecenoic acid; 16:4Δ4,7,10,13, $\Delta^{4,7,10,13}$-all-*cis*-hexadecatetraenoic acid; 18:0, octadecanoic acid (stearic acid); 18:1Δ9, Δ^9-*cis*-octadecenoic acid (oleic acid); 18:1Δ11, Δ^{11}-*cis*-octadecenoic acid; 18:2Δ9,12, $\Delta^{9,12}$-*cis*-*cis*-octadecadienoic acid; 18:3Δ5,9,12, $\Delta^{5,9,12}$-all-*cis*-octadecatrienoic acid; 18:3Δ9,12,15, $\Delta^{9,12,15}$-all-*cis*-octadecatrienoic acid; Chl, chlorophyll; CP, chlorophyll-protein complex; CP II, main light-harvesting chlorophyll *a* + *b*-protein complex in *C. reinhardtii*; DAGTMHS, diacylglyceroltrimethylhomoserine; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DGDG, digalactosyldiacylglycerol; F682, F686, F712, F714, fluorescence emission from whole cells at 77 K showing maximum at 682 nm, 686 nm, 712 nm, 714 nm; LHC, main light-harvesting chlorophyll *a* + *b*-protein complex in higher plants (equivalent to and used in place of LHCP and LHC IIb); MGDG, monogalactosyldiacylglycerol; PC, phosphatidylcholine; PC-di-18:1 or PC-18:1, di-18:1Δ9-containing phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PG-16:1-*trans*, 16:0-16:1-*trans*-containing phosphatidylglycerol; PG-di-16:0, di-16:0-containing phosphatidylglycerol; PI, phosphatidylinositol; Pipes, 1,4-piperazinediethanesulphonic acid; PS I, Photosystem I; PS II, Photosystem II; SL, sulpholipid, sulphoquinovosyldiacylglycerol.

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lar, works by different authors, using various plant systems and various experimental approaches, have suggested that there is a relationship between the presence of 16 : 1-*trans* in PG and the supramolecular organization of the main chlorophyll (Chl) $a + b$ light-harvesting antenna (LHC), especially the formation of oligomeric complexes [5–10]. However, a mutant of *Arabidopsis thaliana,* which lacks 16 : 1-*trans* but shows a normal LHC oligomer content, has been described [11,12]. Concerning the unicellular green alga *Chlamydomonas reinhardtii,* two Photosystem II (PS II)-lacking but low-fluorescent mutants have been isolated and studied in our laboratory. These mutants, *mf* 1 and *mf* 2, are unable to synthesize 16 : 1-*trans,* lack the oligomeric form of the main light-harvesting Chl $a + b$ antenna, CP II, and show anomalies concerning the regulation mechanism of the excitation energy transfer from this antenna towards the photochemical centre regions. In particular, they are unable to carry out an efficient state II-state I transition as shown by low-temperature fluorescence emission spectra [13–15].

Thus, apart from the case of the *A. thaliana* mutant, there are many observations in favour of correlations between the presence of 16 : 1-*trans* in the thylakoid PG, the formation and the stability of oligomeric forms of the main light-harvesting Chl $a + b$ antenna and an efficient regulation of the distribution of the excitation energy captured by this antenna. Nevertheless, the precise function of PG-16 : 1-*trans* is not yet completely elucidated. To go deeper into this question, mutants of unicellular algae can be useful tools and provide interesting information. The present paper reports experiments which were carried out with the aim to obtain an in vivo restoration of the formation of an oligomeric form of the antenna CP II and/or the occurrence of an appreciable fluorescence emission change indicative of state II-state I transition, by incubating cells of the low-fluorescent mutant *mf* 2 of *C. reinhardtii* in the presence of liposomes of PG-16 : 1-*trans*.

Some of the results reported here were presented, in a preliminary and summarized form, at the VIIIth International Congress on Photosynthesis, held August 6–11, 1989, in Stockholm [33].

Materials and Methods

The characteristics of the wild-type of *C. reinhardtii* and of the two mutants *Fl* 39 and *mf* 2 have been described in preceding papers [13–17]. Both these mutants lack PS II; *mf* 2 lacks also 16 : 1-*trans* and is low-fluorescent whereas *Fl* 39, which was used as a control, is a classical high-fluorescent mutant having 16 : 1-*trans*. The algae were grown in the light, in Tris-acetate medium [18] as previously reported [19].

For the experiments, liposomes of PG-16 : 1-*trans,* of palmitate-containing PG (PG-di-16 : 0) or of oleate-containing phosphatidylcholine (PC-di-18 : 1) were prepared by aseptically emulsifying these lipids in culture medium, by means of sonication. PG-16 : 1-*trans* was previously isolated by preparative chromatography from lipid extracts of spinach leaves; PG-di-16 : 0 and PC-di-18 : 1 were commercial chemicals (Sigma). The fatty acid compositions of these different kinds of liposomes are indicated in Table I. PG-di-16 : 0 and PC-di-18 : 1 were pure molecular species. PG-16 : 1-*trans* contained several molecular species having different fatty acids, but the main species was the 16 : 0-16 : 1-*trans*-containing PG (PG-16 : 1-*trans*). The liposomes were added to algae suspensions previously diluted so that their final concentration was $5 \cdot 10^6$ cells $\cdot$ ml^{-1}, permitting a real exponential growth during the experiments. The final lipid concentration in the medium, sufficient for obtaining optimal effects, were of the order of 0.07 mg $\cdot$ ml^{-1} (PG-16 : 1-*trans*), 0.13 mg $\cdot$ ml^{-1} (PG-di-16 : 0) and 0.10 mg $\cdot$ ml^{-1} (PC-di-18 : 1). These suspensions were allowed to incubate at 25 °C in the light with mild shaking, for 39 h before fluorescence measurements and lipid and Chl-protein complex analyses. For some experiments, 1.8 μM cycloheximide (Sigma) was added to the algae suspensions without or together with PG-16 : 1-*trans* liposomes. The incubation time was then reduced to 21 h to avoid grave damage to the cells, as observed after 39 h of incubation in the presence of this antibiotic.

The Chl contents were measured according to Refs. 20 and 21. Lipid analysis was performed using thin layer chromatography on silica-gel plates and capillary gas-liquid chromatography, as described in Refs. 14 and 15. The Chl-protein complexes were analyzed by lithium dodecylsulphate-polyacrylamide gel electrophoresis at 4 °C as previously indicated [14,17] except that, according to Bassi and Simpson [22], a solubilization mixture containing 2% *n*-octyl β-D-glucopyranoside (*n*-octyl β-D-glucopyranoside/Chl = 40 : 1) and 40% glycerol was used. 40% glycerol was also added to the gels. As shown

TABLE I

Fatty acid composition of liposomes of PG-16 : 1-trans, PG-di-16 : 0 and PC-di-18 : 1

Compositions in percentage of total fatty acid contents.

Fatty acids	Liposomes containing		
	PG-16 : 1-*trans* [a]	PG-di-16 : 0 [b]	PC-di-18 : 1 [b]
16 : 0	35.2	100.0	0.0
16 : 1-*trans*	20.4	0.0	0.0
18 : 0	3.3	0.0	0.0
18 : 1Δ9	4.8	0.0	100.0
18 : 2Δ9,12	13.9	0.0	0.0
18 : 3Δ9,12,15	22.4	0.0	0.0

[a] Prepared from spinach leaves.
[b] Commercial chemicals (Sigma).

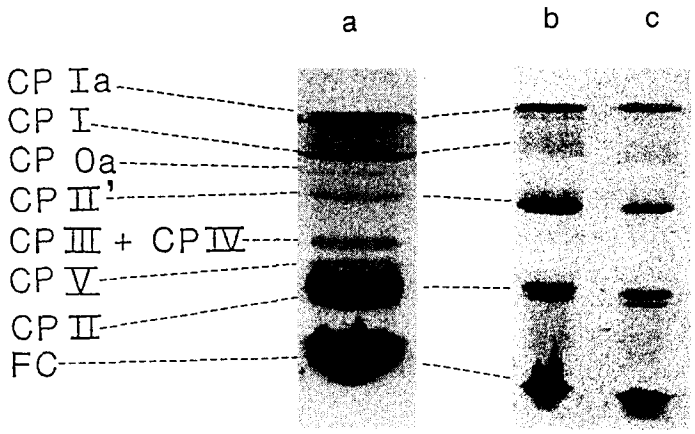

Fig. 1. Effect of two different electrophoretic systems on the isolation of the chlorophyll-protein complexes of *C. reinhardtii*. Chloroplast membranes of the wild-type were solubilized (a) in 0.88% *n*-octyl β-D-glucopyranoside, 0.22% sodium dodecylsulphate, 50 mM dithiothreitol and 20 mM Pipes buffer (pH 6.6) or (b, c) in 2% *n*-octyl β-D-glucopyranoside, 40% glycerol, 50 mM dithiothreitol and 20 mM Pipes buffer (pH 6.6). Lithium dodecylsulphate-polyacrylamide gel electrophoreses were then performed without (a) or after (b, c) addition of 40% glycerol to the 11% polyacrylamide gels. Membrane concentrations on the gels: 30 (a), 10 (b) and 4 (c) μg of Chl per well. Unstained gel: all the bands were green pigmented. FC, free chlorophyll. Note that in (b) and (c), despite much lower membrane concentrations, the bands of CP II′ were of greater importance than in (a).

in Fig. 1, lanes b and c, this method allowed us to isolate CP II′, the oligomeric form of CP II, with a yield clearly higher than by using 0.88% *n*-octyl β-D-glucopyranoside and 0.22% sodium dodecylsulphate (lane a). On the other hand, it did not permit suitable isolation of the different complexes CP I, CP 0 and CP 0a of Photosystem I (PS I) and CP III, CP IV and CP V of PS II. Fluorescence emission spectra of cells were measured at 77 K as described in Ref. 23. State I conditions (oxidized plastoquinone pool) were obtained by aeration of the cell suspensions, state II conditions (reduced plastoquinone pool) by incubating the cell suspensions in the presence of 5 mM NaN_3 according to Ref. 24.

Results

Lipid incorporation

Kinetics of the incorporation of PG-16 : 1-*trans* liposomes into chloroplasts of *mf* 2 are shown in Fig. 2. Both the total PG content in chloroplasts and the 16 : 1-*trans* content in PG showed maximum values after 36 h of incubation then decreased, whereas the PG-16 : 1-*trans* content in medium continued to decrease up to 48 h. This probably indicates a relatively rapid turnover of these lipids. Finally, an incubation time of 39 h was chosen for the experiments in order to allow a maximum of cell development and divisions to occur. The polar lipid compositions of the mutants *Fl* 39 and *mf* 2, without and after incubation in the presence of PG-16 : 1-*trans* liposomes, are summarized in the four first columns of Table II. The PG content of *mf* 2 was very low in untreated cells, corresponding to 1.6% of total polar lipids. It was clearly increased after incubation of the cells in the presence of PG-16 : 1-*trans*, reaching to a content value comparable to that of *Fl* 39 and to a proportion of 8.8% of total polar lipids.

Table III indicates the fatty acid compositions of cells of the *Fl* 39 and *mf* 2 controls and of *mf* 2 incubated in the presence of the different kinds of liposomes. These compositions showed about equal amounts of a C_{16} series and a C_{18} series, the more abundant fatty acids being 16 : 0, 16 : 4Δ4,7,10,13 and 18 : 3Δ9,12,15. They were comparable to the compositions previously observed by us for the wild-type and the mutants *Fl* 39 and *mf* 2 [14] and by Giroud et al. for the wild-type [25]. The composition of *mf* 2 was not very different from that of *Fl* 39 except that 16 : 1-*trans* was totally missing in the former mutant. After incubation of the cells in the presence of PG-16 : 1-*trans* liposomes, an appreciable amount of 16 : 1-*trans*, comparable to that of *Fl* 39, was found in *mf* 2. On the other hand, the proportion of 16 : 0 was greatly increased for *mf* 2, confirming the incorporation of an appreciable part of the PG-16 : 1-*trans* provided by the liposomes. The results relative to cells of *mf* 2 which had been incubated in the presence of liposomes of PG-di-16 : 0 and PC-di-18 : 1 (Table III) indicated that these lipids have been really incorporated into the cells. No 16 : 1-*trans* was detected in these samples.

Profiles of analysis of the PG fatty acids from the wild-type (A) and the mutants *Fl* 39 (B) and *mf* 2 (C, D) are shown in Fig. 3. These profiles confirmed the absence of 16 : 1-*trans* in the PG of *mf* 2 (C) and its presence in the PGs of the wild-type (A) and of *Fl* 39

TABLE II

Polar lipid composition of cells of the mutants Fl 39 and mf 2 of C. reinhardtii, without and after incubation in the presence of liposomes of PG-16:1-trans, in the absence and in the presence of cycloheximide

Contents in μg of fatty acids per mg of chlorophyll. Liposomes were added to the culture medium then the algae suspensions were allowed to grow at 25°C in the light with mild shaking, for 39 h (*Fl* 39, *mf* 2) or 21 h (*mf* 2). The lipid classes were analyzed by thin layer chromatography on silica gel plate. Solvents: chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5, v/v). The amounts of lipids were then determined by densitometry. For *mf* 2 incubated in the absence of cycloheximide, the contents were similar after 39 h (not shown) and after 21 h of incubation. Cyclohex., cycloheximide.

Lipid classes	Mutants					
	Fl 39		*mf* 2			
	control	+ PG-16:1-*trans* [a]	control	+ PG-16:1-*trans* [a]	+ cyclohex. [b]	+ cyclohex. + PG-16:1-*trans*
PI	30	40	23	26	12	12
SL [c]	88	79	112	125	60	50
DGDG	90	137	193	194	139	126
PG	95	100	20	111	12	47
PE	130	120	224	198	125	97
DAGTMHS	389	363	434	378	238	236
MGDG	179	164	216	228	165	169
Total polar lipids	1001	1003	1222	1260	751	737

[a] 0.07 $mg \cdot ml^{-1}$ of algae suspension.
[b] 1.8 μM.
[c] SL was shown to contain 5% of phosphatidylcholine.

TABLE III

Fatty acid composition of cells of the mutants Fl 39 and mf 2 of C. reinhardtii, without and after incubation in the presence of liposomes of PG-16:1-trans, PG-di-16:0 and PC-di-18:1 for 39 h

Compositions in percentage of total fatty acid contents. Liposomes were added to the culture medium then the algae suspensions were allowed to grow for 39 h at 25°C in the light with mild shaking. The fatty acids of the total lipid extracts were transmethylated then analyzed by capillary gas-liquid chromatography.

Fatty acids	Mutants				
	Fl 39	*mf* 2			
	control	control	+ PG-16:1 *trans* [a]	+ PG-di-16:0 [b]	+ PG-di-18:1 [c]
16:0	21.1	20.5	31.1	30.1	13.0
16:1Δ9	7.2	11.4	7.1	7.8	11.0
16:1-*trans*	2.2	0.0	1.9	0.0	0.0
16:4Δ4,7,10,13	18.8	15.9	10.9	15.5	9.3
18:0	3.3	3.9	4.1	4.7	1.1
18:1Δ9	2.8	6.7	6.2	9.3	26.5
18:1Δ11	4.0	7.6	4.4	4.4	0.3
18:2Δ9,12	7.3	4.9	7.6	5.1	17.4
18:3Δ5,9,12	6.2	6.3	4.4	7.2	6.2
18:3Δ9,12,15	27.1	22.8	22.3	15.9	15.2

[a] 0.07 $mg \cdot ml^{-1}$ of algae suspension.
[b] 0.13 $mg \cdot ml^{-1}$ of algae suspension.
[c] 0.10 $mg \cdot ml^{-1}$ of algae suspension.

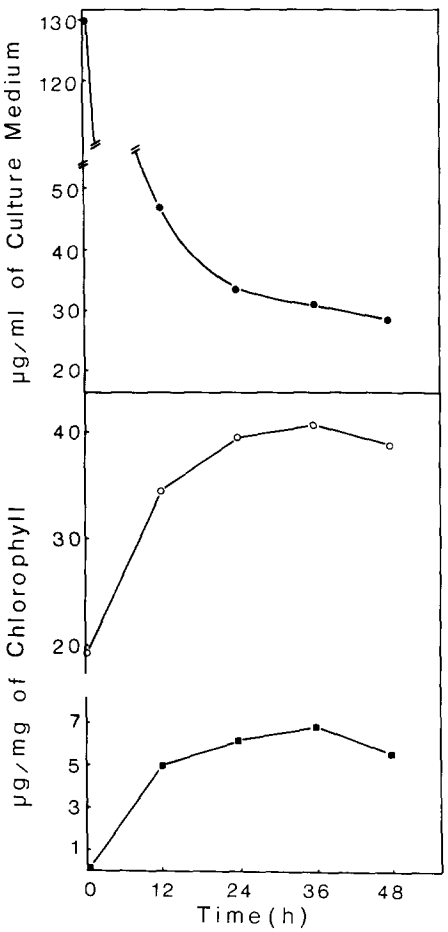

Fig. 2. Kinetics of PG-16:1-*trans* incorporation into chloroplasts of the mutant *mf* 2 of *C. reinhardtii*. The cells were incubated in the presence of liposomes of PG-16:1-*trans* dispersed in culture medium, at 25 °C in the light with mild shaking, for different times before lipid analyses. ●, PG-16:1-*trans* in culture medium (µg/ml of culture medium); ○, PG in chloroplast pellet (µg/mg of chlorophyll); ■, 16:1-*trans* in chloroplast PG (µg/ml of chlorophyll). In this preliminary experiment, the initial concentration of PG-16:1-*trans* in culture medium was 1.8-times higher than the concentration afterwards commonly used in the restoration experiments.

(B). They showed clearly the appearance of a peak of 16:1-*trans* in the PG of *mf* 2 after incubation of cells of this mutant in the presence of PG-16:1-*trans* liposomes (D) for 21 h (a similar profile was obtained after incubation for 39 h).

Chlorophyll-protein complexes

Electrophoretograms of the Chl-protein complexes of chloroplast membranes from the wild-type and from the mutants *Fl* 39 and *mf* 2 are shown in Fig. 4. The electrophoretogram of the wild-type (lane a) shows the following bands: CP Ia corresponding to the undissociated PS I complex, CP I corresponding to the core antenna and the reaction center of PS I, CP II and CP II′ corresponding respectively to the monomeric and the oligomeric forms of the main light-harvesting Chl *a* + *b*-protein antenna [13,14]. The bands of CP III, CP IV and CP V, which correspond to the reaction centre of PS II and to its core and peripheral antennae, did not appear on this kind of electrophoretogram (see Materials and Methods).

For the PS II-lacking mutant *Fl* 39 (lanes b, f) the same bands as for the wild-type were observed, in particular an important band of CP II′. On the other hand, no CP II′ was seen for the mutant *mf* 2 (lane c), as previously reported [13,14]. However, it must be noted that traces of CP II′, scarcely visible, have sometimes been observed on other electrophoretograms of *mf* 2, indicating that CP II oligomer can probably be formed but not accumulated in this mutant. In contrast,

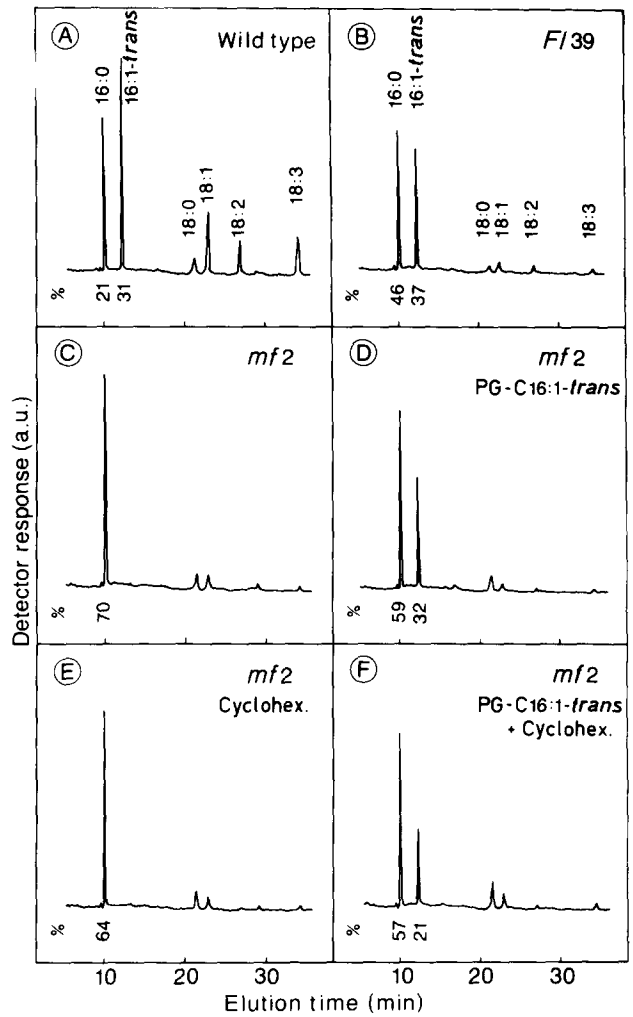

Fig. 3. Fatty acid composition of the PGs from the wild-type (A) and the mutants *Fl* 39 (B) and *mf* 2 (C–F) of *C. reinhardtii*, without (A–C) and after incubation of the cells in the presence of PG-16:1-*trans* liposomes (D), of cycloheximide (E) and of both PG-16:1-*trans* liposomes and cycloheximide (F) for 21 h. The fatty acids from PG, which had been isolated by thin-layer chromatography, were transmethylated and then analyzed by capillary gas-liquid chromatography. The percentages indicate the relative importance of the areas of the peaks corresponding to 16:0 and to 16:1-*trans*. a.u., arbitrary unit; cyclohex., cycloheximide.

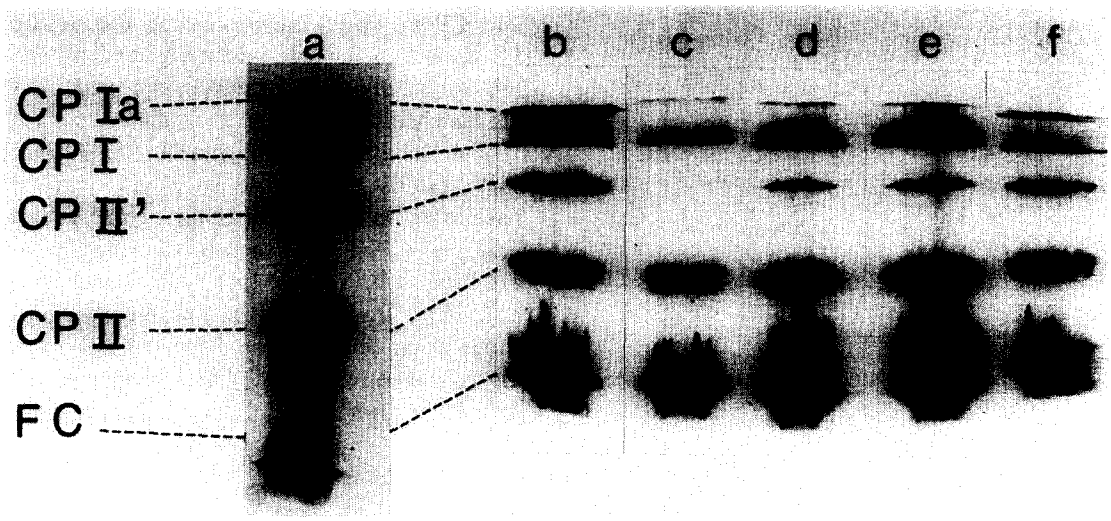

Fig. 4. Chlorophyll-protein complexes of the wild-type (a) and the mutants *Fl* 39 (b, f) and *mf* 2 (c–e) of *C. reinhardtii*, without (a–c) and after (d–f) incubation of the cells in the presence of PG-16 : 1-*trans* liposomes for 39 h. Chloroplast membranes were solubilized, at a final concentration of 0.5 mg of Chl·ml^{-1}, in 2% *n*-octyl β-D-glucopyranoside, 40% glycerol, 50 mM dithiothreitol and 20 mM Pipes buffer (pH 6.6). Lithium dodecylsulphate polyacrylamide gel electrophoresis was then performed using 3% and 11% polyacrylamide gels to which 40% glycerol was added. Membrane concentrations on the gels: 11 (a, b), 9 (c, f), 12 (d) and 18 (e) μg of Chl per well. Unstained gel: all the bands were green pigmented. FC, free chlorophyll.

an appreciable band of CP II′ clearly appeared when the cells of *mf* 2 had been incubated in the presence of PG-16 : 1-*trans* liposomes (lanes d, e). In the case of cells of *Fl* 39 incubated in the presence of PG-16 : 1-*trans* liposomes (lane f), the same complexes as without PG-16 : 1-*trans* incubation (lane b) were observed.

Electrophoreses were also performed with chloroplast membranes of *mf* 2, the cells of which had been incubated in the presence of liposomes of either PG-di-16 : 0 or PC-di-18 : 1. No CP II′ was detected in the case of PC-di-18 : 1 and only some traces in that of PG-di-16 : 0 (not shown).

Low-temperature fluorescence emission spectra

Fluorescence emission spectra, measured with cells of the different samples which had been frozen in state I and in state II conditions, are shown in Fig. 5. An excitation light of $\lambda = 475$ nm was used, corresponding to a radiation preferentially absorbed by Chl *b* and consequently by the light-harvesting antenna CP II. As previously described [13,23], several peaks or shoulders can be observed on these spectra. They reflect different emission bands: F_{682}, in the 682 nm region, related to the main light-harvesting antenna CP II; F_{686} and F_{696}, in the 686 nm and 696 nm regions, related to the complexes CP IV and CP III which correspond to the antenna and to the core of PS II, respectively; F_{712} or F_{714}, in the 712–714 nm region, related to the complex CP I which corresponds to the core of PS I (core antenna and reaction center). On the spectra of the wild-type, the emissions F_{686}, F_{696} and F_{714} appeared. In state II (reduced plastoquinone pool, dashed line), F_{686} was only slightly more important than F_{714} ($F_{686}/F_{714} = 1.12$) whereas in state I (oxidized plastoquinone pool, solid line) the difference was greater ($F_{686}/F_{714} = 1.95$).

In the case of the mutant *Fl* 39, there was no emission at 686 nm and 696 nm but F_{682} was clearly observed. Indeed, the antenna CP II was unable to transfer a part of its excitation energy towards PS II, which was missing, and emitted its own fluorescence. This emission was not very important in state II (dashed line, $F_{682}/F_{714} = 0.31$). It became clearly increased in state I (solid line), F_{682}/F_{712} (0.97) being 3-fold higher. These spectra relative to the wild-type and to the mutant *Fl* 39 were comparable to those reported by Wollman and Delepelaire for the wild-type and another PS-II-lacking mutant, F 34, of *C. reinhardtii* [24]. They illustrate typical state II-state I transitions in the distribution of the excitation energy among the two photosystem regions. The regulation of this energy distribution involves a phosphorylation-mediated migration of a fraction of the main light-harvesting antenna. It is controlled by the oxido-reduction level of the plastoquinone pool (see Ref. 26 for a review).

The mutant *mf* 2 also lacks PS II but its spectra were different from those of the mutant *Fl* 39. Indeed, even if a weak state II-state I transition occurred, the emission in the 682 nm region was very low after both the treatments aimed at inducing state II (dashed line, $F_{682}/F_{712} = 0.12$) and state I (solid line, $F_{682}/F_{712} = 0.20$). This indicates a highly predominant energy transfer towards PS I, whatever the oxido-reduction level of the plastoquinone pool. On the other hand, when the cells of *mf* 2 had been incubated in the presence of PG-16 : 1-*trans* liposomes (*mf* 2 PG-16 : 1-*trans*), a peak

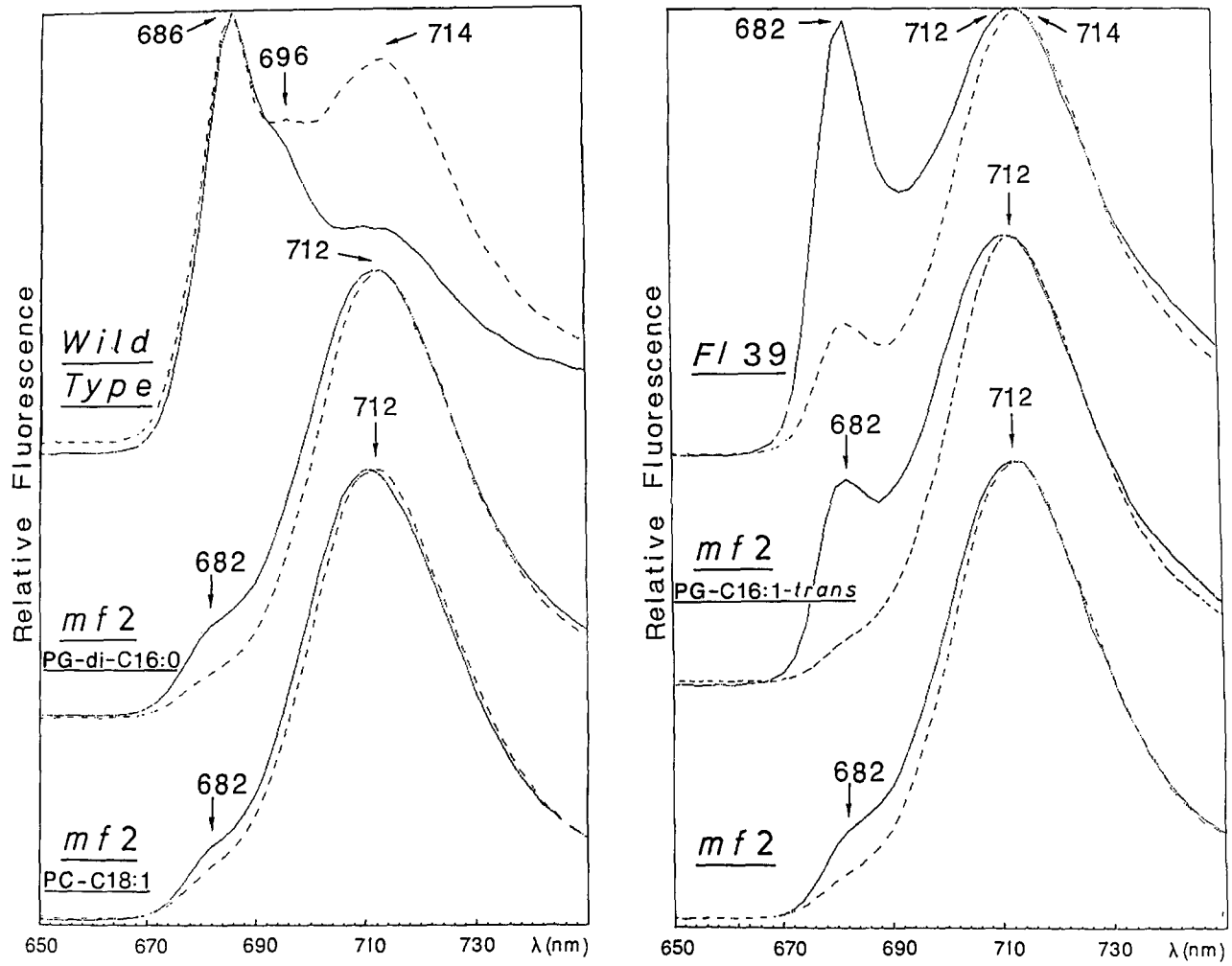

Fig. 5. Low-temperature fluorescence emission spectra of cells of the wild type and of the mutants *Fl* 39 and *mf* 2 of *C. reinhardtii* in state I (solid lines) and in state II (dashed lines), without (wild type, *Fl* 39, *mf* 2) and after (*mf* 2) incubation in the presence of liposomes of PG-di-16:0, PC-18:1 and PG-16:1-*trans* for 39 h. The cells (40 μg of Chl·ml^{-1}) were suspended in phosphate buffer (pH 7.5) and either aerated at room temperature in the light with mild shaking, in the presence of 10 μM DCMU in the case of the wild-type, for 25 min (state I) or incubated in the presence of 5 mM NaN_3 in the dark at room temperature for 40 min (state II). The cell suspensions were then frozen in 0.1 mm thick cuvettes, which were set against the front of the optical guide of the spectrofluorimeter and plunged into liquid nitrogen. Excitation light λ = 475 nm. Slit of the analytical monochromator, 2 nm. The spectra were normalized at their respective maxima. The numbers indicate the λ of the peaks and shoulders.

at 682 nm clearly appeared in state I conditions (solid line). So the ratio F_{682}/F_{712} (0.46) was more than 2-fold increased and was 3.8-times higher than in state II conditions (0.12), indicating the restoration of an appreciable state II-state I transition. Such a restoration was constantly observed during nine other independent experiments using various PG-16:1-*trans* concentrations. No comparable restoration of an appreciable state II-state I transition occurred when *mf* 2 cells has been incubated in the presence of liposomes of PG-dipalmitate (*mf* 2 PG-di-16:0, $F_{682}/F_{712} = 0.12$ and 0.23) or of PC-dioleate (*mf* 2 PC-18:1, $F_{682}/F_{712} = 0.13$ and 0.17).

Effect of cycloheximide

Cells of the mutant *mf* 2 were incubated for 21 h in the presence of both PG-16:1-*trans* liposomes and cycloheximide. This antibiotic blocks the cytoplasmic protein synthesis on 80 S ribosomes and consequently the synthesis of CP II apoproteins. Indeed, these apoproteins are synthesized on the cytoplasmic ribosomes as higher-molecular weight pre-apoproteins, which are then assembled in the chloroplast (see Ref. 27).

The polar lipid compositions of the cells incubated in the presence of cycloheximide are indicated in the two last columns of Table II. The lipid contents were less important than those of the cells grown in the absence of antibiotic. This is probably due to the non-occurrence of cell growth in the presence of cycloheximide. Nevertheless, it clearly appears that cycloheximide did not prevent the incorporation of PG-16:1-*trans* into the cells of *mf* 2. Indeed, the PG content was 3.9-times

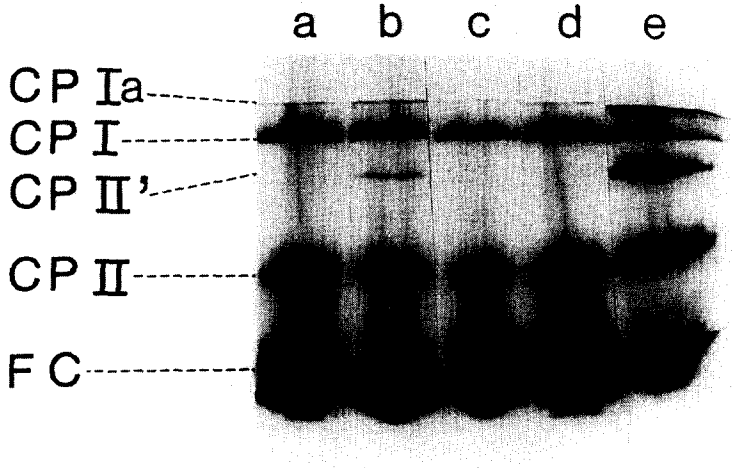

Fig. 6. Chlorophyll-protein complexes of the wild-type (e) and the mutant *mf* 2 (a–d) of *C. reinhardtii*, without (a, e) and after incubation of the cells in the presence of PG-16 : 1-*trans* liposomes (b), of cycloheximide (c) and of both PG-16 : 1-*trans* liposomes and cycloheximide (d) for 21 h. The chloroplast membranes were solubilized then analyzed as indicated in the legend of Fig. 4. Membrane concentrations on the gel: 20 (a–c, e) and 25 (d) μg of Chl per well. These high concentrations, necessary for detecting low amounts of CP II′, were responsible for the relatively low quality of the electrophoretograms. Unstained gel: all the bands were green-pigmented. FC, free chlorophyll.

higher for the cells incubated in the presence of cycloheximide and PG-16 : 1-*trans* liposomes than for those incubated in the presence of cycloheximide only.

The electrophoretograms of Fig. 6 show that a band of CP II′ was clearly observed for the wild-type (lane e) and for *mf* 2 incubated in the presence of PG-16 : 1-*trans* (lane b). On the other hand, as in the case of untreated *mf* 2 (lane a) and of *mf* 2 incubated in the presence of cycloheximide only (lane c), no CP II′ was detected when *mf* 2 cells had been incubated in the presence of both cycloheximide and PG-16 : 1-*trans* liposomes (lane d). Besides, it was also verified that the amount of CP II′ in the membranes of the mutant *Fl* 39 was not greatly modified after incubation of cells of this mutant in the presence of cycloheximide or of both cycloheximide and PG-16 : 1-*trans* liposomes for 21 h (not shown).

Low-temperature fluorescence emission spectra of cells of the mutants *Fl* 39 and *mf* 2 are shown in Fig. 7. The spectra of cells of *Fl* 39 incubated in the presence of both cycloheximide and PG-16 : 1-*trans* liposomes showed an important state II-state I transition: F_{682}/F_{712} in state I (1.32, solid line) was 2.8-times higher than F_{682}/F_{714} in state II (0.47, dashed line). As already observed, the spectra of the mutant *mf* 2 only indicated a very weak state II-state I transition, F_{682}/F_{712} being 0.16 in state II (dashed line) and 0.23 in state I (solid line) conditions. After incubation of *mf* 2 cells in the presence of PG-16 : 1-*trans* liposomes (*mf* 2 PG-16 : 1-*trans*), an appreciable state II-state I transition was restored, F_{682}/F_{712} being 2.2-times higher in state I (0.39, solid line) than in state II (0.18, dashed line). On the other hand, it clearly appears that such a restoration did not occur when *mf* 2 cells had been incubated in the presence of both PG-16 : 1-*trans* and cycloheximide (*mf* 2 PG-16 : 1-*trans* + cyclohex.). Indeed, in this case, the curves corresponding to state II and to state I conditions were practically superimposed, only showing a very weak shoulder in the 682 nm region.

Thus, cycloheximide prevented both the formation of the oligomer CP II′ and the occurrence of a fluorescence state II-state I transition in *mf* 2 cells incubated in the presence of PG-16 : 1-*trans*. This indicates that the novo synthesis of CP II apoproteins was needed for the restoration of these processes. However, cycloheximide inhibited neither the 16 : 1-*trans* incorporation in the thylakoid PG of *mf* 2 nor the fluorescence state II-state I transition in *Fl* 39, which contains 16 : 1-*trans* and CP II′.

Discussion

The present results pointed out a clear correlation between the presence of 16 : 1-*trans* in PG, the presence of an oligomeric form of the main light-harvesting Chl $a + b$ antenna, CP II, and the occurrence of a state II-state I transition in the regulation of the excitation energy distribution between this antenna and PS I. This correlation is not a simple coincidence as shown by the double restoration of both the presence of CP II oligomer and the occurrence of a state II-state I transition when PG-16 : 1-*trans*, and exclusively this lipid, was incorporated into the thylakoid membrane.

In the case of higher plants, much evidence exists that the main light-harvesting antenna (LHC) occurs in

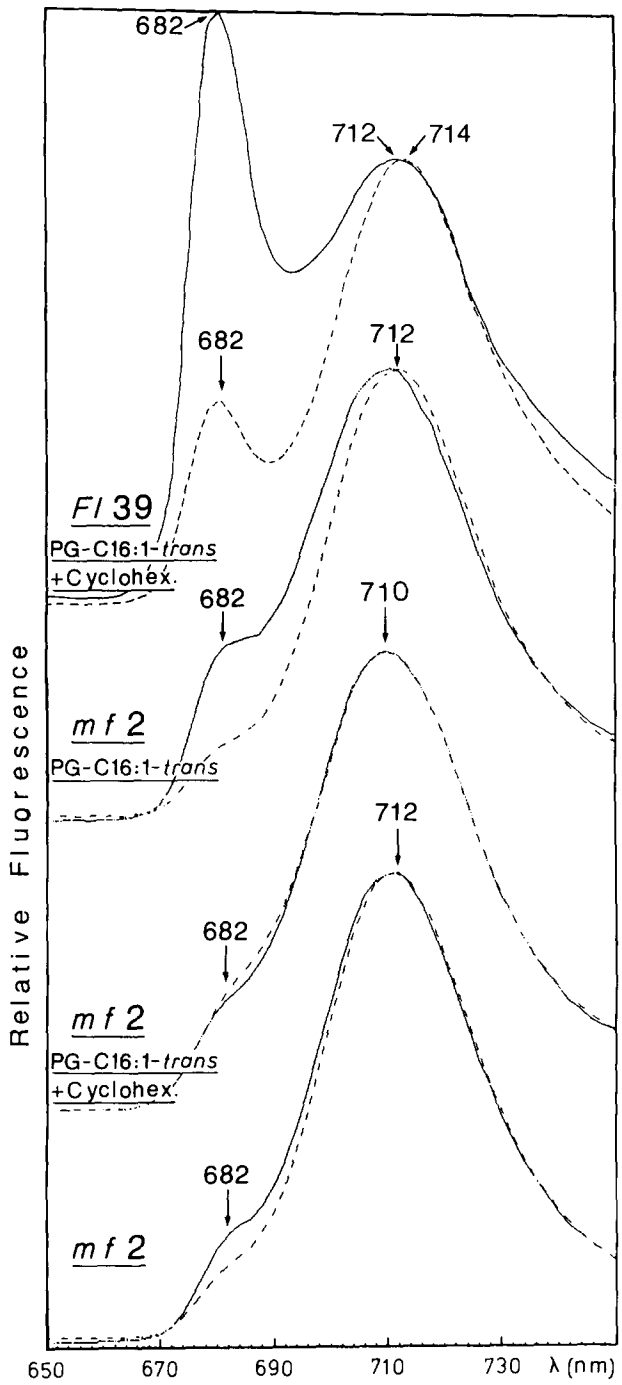

Fig. 7. Low-temperature fluorescence emission spectra of cells of the mutants *Fl* 39 and *mf* 2 of *C. reinhardtii* in state I (solid lines) and in state II (dashed lines), without (*mf* 2) and after incubation in the presence of PG-16 : 1-*trans* liposomes (*mf* 2) and of both PG-16 : 1-*trans* liposomes and cycloheximide (*Fl* 39, *mf* 2) for 21 h. The spectra were measured as indicated in the legend of Fig. 5; they were normalized at their peaks in the 710–714 nm region. The numbers indicate the λ of the peaks and shoulders. Cyclohex., cycloheximide.

vivo in an oligomeric form [27], which corresponds to a trimer [28]. No data relative to this question and concerning green algae was found in the literature. Our present findings concerning *C. reinhardtii* indicate that an oligomeric form of CP II plays a real role in vivo. Indeed, this form appears necessary, in state I conditions, for the occurrence of an important energy transfer towards PS II, in the wild-type, or for a strong emission of the own fluorescence of CP II when PS II is missing as in the mutants *Fl* 39 and *mf* 2. Thus, an oligomeric organization would be essential to an efficient adhesion of the bulk of CP II to the PS II regions, eventually in correlation with thylakoid appression. On the other hand, CP II in its monomeric form would easily adhere to the PS I regions. The relationship between this essential role of the oligomeric form of CP II and the mechanism of the state II-state I regulation of the energy distribution, mechanism which involves the dephosphorylation of a mobile fraction of the antenna having a specific polypeptide composition [29], remains to be elucidated. It is probable that these two processes, even if they can be involved together, do not occur at the same level of organization.

To find appreciable amounts of CP II oligomer and observe an appreciable state II-state I transition in the mutant *mf* 2, it was necessary to supply its cells with 16 : 1-*trans* by means of an incubation in the presence of liposomes of PG-16 : 1-*trans*. Thus it appears that 16 : 1-*trans* esterified in PG plays an essential role in the stabilization and the accumulation of the oligomeric form of CP II. Moreover, de novo synthesis of apoproteins of CP II is also needed for the restoration of both a CP II oligomer and a state II-state I transition. This rules out the eventuality that PG-16 : 1-*trans* only participates in vivo in a simple association, through weak interactions, between pre-existing molecules of CP II monomer, as probably occurring in vitro (see Ref. 8).

These results do not agree with those of Browse et al. [11] and McCourt et al. [12] which have described a mutant of *Arabidopsis thaliana* lacking 16 : 1-*trans* but showing LHC oligomeric form in amounts comparable to those of the wild-type. This mutant did not show anomalies concerning the capture and the distribution of light energy. However, its oligomeric form of LHC appeared more labile towards detergent-mediated dissociation than that of the wild-type. On the other hand, our findings fully agree with those of Huner et al. [30] and Krol et al. [31]. Studying chloroplasts of *Secale cereale,* these authors have pointed out a clear correlation between decrease in the 16 : 1-*trans* content of PG and decrease in the ratio of the oligomeric to the monomeric forms of LHC, when the seedlings were grown at 5 °C as compared to 20 °C. They have concluded that the presence of 16 : 1-*trans* in PG is not obligatory but probably enhances the stabilization of oligomeric LHC. The fluorescence data reported by these authors [32], which concern structural alterations in LHC and PS II reaction centre but not state II-state I transition, cannot be significantly compared to our present data.

Taking into account all these observations, we can draw the following conclusions: (1) in *C. reinhardtii*, the oligomeric form of the main Chl $a + b$ light-harvesting antenna, CP II, is essential to a good excitation energy transfer towards PS II and, consequently, to a good state II-state I transition in the distribution of the excitation energy among the photosystems; (2) the genesis of this oligomeric form of CP II involves the assembly of new Chl $a + b$-protein complexes in the chloroplast and, consequently, needs the cytoplasmic synthesis of apoprotein precursors; (3) during this CP II assembly, PG-16 : 1-*trans* plays an essential role in stabilizing the neo-formed oligomers and, probably, in making easier their arrangement in the membrane; (4) in the absence of 16 : 1-*trans* in PG, the oligomer of CP II can probably be formed but it is unstable and becomes rapidly dissociated.

Experiments are in progress to study the eventual relationships between PG-16 : 1-*trans*, CP II oligomer and thylakoid stacking.

Acknowledgements

The authors thank Mrs. A. Bennardo-Connan, Mrs. A. Trouabal and M.R. Boyer, whose excellent technical assistance was greatly appreciated.

References

1 Rawyler, A. and Siegenthaler, P.A. (1981) Biochim. Biophys. Acta 638, 30–39.
2 Unitt, M.D. and Harwood, J.L. (1985) Biochem. J. 228, 707–711.
3 Dubacq, J.-P. and Trémolières, A. (1983) Physiol. Vég. 21, 293–312.
4 Siegenthaler, P.A., Smutny, J. and Rawyler, A. (1987) Biochim. Biophys. Acta 891, 85–93.
5 Trémolières, A., Dubacq, J.-P., Ambard-Bretteville, F. and Rémy, R. (1981) FEBS Lett. 130, 27–31.
6 Trémolières, A., Dubacq, J.-P., Duval, J.-C., Lemoine, Y. and Rémy, R. (1982) in Biochemistry and Metabolism of Plant Lipids (Wintermans, J.F.G.M. and Kuiper, P.J.C., eds.), pp. 369–372, Elsevier, Amsterdam.
7 Lemoine, Y., Dubacq, J.-P. and Zabulon, G. (1982) Physiol. Vég. 20, 487–503.
8 Rémy, R., Trémolières, A. and Ambard-Bretteville, F. (1984) Photobiochem. Photobiophys. 7, 267–276.
9 Huner, N.P.A., Krupa, Z., Williams, J.P. and Maissan, E. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 4, pp. 119–122, Martinus Nijhoff, Dordrecht.
10 Krupa, Z., Huner, N.P.A., Williams, J.P., Maissan, E. and James, D.R. (1987) Plant Physiol. 84, 19–24.
11 Browse, J., McCourt, P. and Somerville, C.R. (1985) Science 227, 763–765.
12 McCourt, P., Browse, J., Watson, J., Anrtzen, C.J. and Somerville, C.R. (1985) Plant Physiol. 78, 853–858.
13 Garnier, J., Maroc, J. and Guyon, D. (1987) Plant Cell Physiol. 28, 1117–1131.
14 Maroc, J., Trémolières, A., Garnier, J. and Guyon, D. (1987) Biochim. Biophys. Acta 893, 91–99.
15 Seras, M., Garnier, J., Trémolières, A. and Guyon, D. (1989) Plant Physiol. Biochem. 27, 393–399.
16 Garnier, J., Guyon, D. and Picaud, A. (1979) Plant Cell Physiol. 20, 1013–1027.
17 Maroc, J., Guyon, D. and Garnier, J. (1983) Plant Cell Physiol. 24, 1217–1230.
18 Gorman, D.S. and Levine, R.P. (1965) Proc. Natl. Acad. Sci. USA 54, 1665–1669.
19 Garnier, J. and Maroc, J. (1972) Biochim. Biophys. Acta 283, 100–114.
20 MacKinney, G. (1941) J. Biol. Chem. 140, 315–322.
21 Gaudillère, J.P. (1974) Physiol. Vég. 12, 585–599.
22 Bassi, R. and Simpson, D. (1987) in Progress in Photosynthesis Research (Biggins, J., ed.), Vol. 2, pp. 81–88, Martinus Nijhoff, Dordrecht.
23 Garnier, J., Maroc, J. and Guyon, D. (1986) Biochim. Biophys. Acta 851, 395–406.
24 Wollman, F.-A. and Delepelaire, P. (1984) J. Cell Biol. 98, 1–7.
25 Giroud, C., Gerber, A. and Eichenberger, W. (1988) Plant Cell Physiol. 29, 587–595.
26 Iordanov, I.T. and Goltsev, V.N. (1987) Photosynthetica 21, 236–250.
27 Thornber, J.P., Peter, G.F., Chitnis, P.R., Nechushtai, R. and Vainstein, A. (1988) in Light-Energy Transduction in Photosynthesis: Higher Plant and Bacterial Models (Stevens Jr., S.E. and Bryant, D.A., eds.), pp. 137–154, The American Society of Plant Physiologists, Rockville.
28 Kühlbrandt, W. (1988) J. Mol. Biol. 202, 849–864.
29 Bassi, R., Giacometti, G.M. and Simpson, D.J. (1988) Biochim. Biophys. Acta 935, 152–165.
30 Huner, N.P.A., Krol, M., Williams, J.P., Maissan, E., Low, P.S., Roberts, D. and Thompson, J.E. (1987) Plant Physiol. 84, 12–18.
31 Krol, M., Huner, N.P.A., Williams, J.P. and Maissan, E. (1988) Photosynth. Res. 15, 115–132.
32 Griffith, M., Huner, N.P.A. and Kyle, D.J. (1984) Plant Physiol. 76, 381–385.
33 Garnier, J., Wu, B., Maroc, J., Guyon, D. and Trémolières, A. (1990) in Current Research in Photosynthesis (Baltscheffsky, M., ed.), Vol. II, pp. 277–280, Kluwer, Dordrecht.