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# Low-temperature fluorescence emission spectra and chlorophyll-protein complexes in mutants of *Chlamydomonas reinhardtii*: evidence for a new chlorophyll-*a*-protein complex related to Photosystem I

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**With the aim of confirming the relationships between low-temperature emission bands of chlorophyll fluorescence of whole cells and chlorophyll-protein complexes as defined by their electrophoretic behaviour, a study was performed with the wild type of *Chlamydomonas reinhardtii* and eight mutants which lacked one or several chlorophyll-protein complexes and showed impaired photosynthetic functions. Chlorophyll-protein complexes from chloroplast fragments or Triton X-100-treated particles, which were enriched in both Photosystems I and II (PS I and PS II), were analyzed by lithium dodecylsulfate polyacrylamide gel electrophoresis at 4°C, using either lithium dodecylsulfate or *n*-octyl-β-D-glucopyranoside in the solubilization mixtures. Absorption and fluorescence emission spectra were measured at 77 K with whole cells and also, for fluorescence, with isolated chlorophyll-protein complexes. Fluorescence emissions in the 680–682 nm ($F_{682}$), 686 nm ($F_{686}$), 696 nm ($F_{696}$), 703 nm ($F_{703}$), 707 nm ($F_{707}$) and 712–717 nm ($F_{715}$) regions were observed with whole cells of the different strains. A comparative study of the chlorophyll-protein complexes, the fluorescence emissions and the photochemical activities shown by each strain confirmed the following correlations, which have been previously described or proposed in the literature for algae or higher plants: $F_{682}$ with CP II, the chlorophyll (Chl) $a + b$ complex corresponding to the main light-harvesting antenna; $F_{686}$ and $F_{696}$ with CP IV and CP III, the Chl *a* complexes corresponding to the antenna and to the core of PS II respectively; $F_{707}$ with CP 0, the Chl $a + b$ complex part of the PS I antenna; $F_{715}$ with CP I, the Chl *a* complex corresponding to the core of PS I. A new PS-I-related Chl *a*-protein complex, tentatively designated CP 0a, was also observed. This complex showed an apparent relative molecular mass slightly smaller than that of CP I. It was the only PS I-related complex in the double mutant *Fl* 5 *Pg* 27 which lacks Chl *b*, CP 0, CP I and CP II. It appeared to be correlated with $F_{703}$, which was observed with cells of the same double mutant. The emission spectrum of another mutant, *Fl* 50 which contained CP I and CP II but was deficient in CP 0 and CP 0a, showed a $F_{715}$ contribution significantly reduced, indicating that CP 0 and CP 0a play an essential part in energy transfer from CP II to CP I. The probable pathways of light energy transfer in *C. reinhardtii* were examined. It is proposed that CP 0a acts as a connecting antenna between CP 0 and CP I, the energy transfer from CP II to PS I occurring through the following sequence: CP II → CP 0 → CP 0a → CP I core antenna → PS I reaction center.**

Abbreviations: Chl *a*, chlorophyll *a*; Chl *b*, chlorophyll *b*; CP, chlorophyll-protein complex; DCIP, 2,6-dichlorophenolindophenol; $DCIPH_2$, reduced DCIP; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; $F_{682}$, $F_{685}$, $F_{707}$ etc., fluorescence emission from whole cells at 77 K showing maximum at 682 nm, 685 nm, 707 nm etc.; LDS, lithium dodecylsulfate; SDS, sodium dodecylsulfate; LHC, light-harvesting chlorophyll *a*/*b*-protein complex; Pipes, 1,4-piperazinediethanesulfonic acid; PS I, Photosystem I; PS II, Photosystem II; P-680, chlorophyll *a* holochrome, active pigment in PS II; P-700, chlorophyll *a* holochrome, active pigment in PS I.

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## Introduction

In thylakoid membranes of algae and higher plants, chlorophylls are associated with protein chains, forming several chlorophyll-protein complexes which have different analytic, spectroscopic and functional characteristics. These complexes show specific absorbance properties in vivo, as pointed out by numerous studies which have been performed with whole organisms or chloroplast fractions using spectra analysis methods. These studies have described different chlorophyll *a* (Chl *a*) forms, the short-wavelength red-absorbing forms being predominantly present in Photosystem II (PS II) and in light-harvesting antennae, the long-wavelength red-absorbing forms being predominantly present in Photosystem I (PS I) (see Refs. 1–3). Concerning chlorophyll fluorescence, five main emission bands near 680 nm ($F_{680}$), 685 nm ($F_{685}$), 695 nm ($F_{695}$), 720 nm ($F_{720}$) and 735 nm ($F_{735}$) have been observed with higher plant chloroplasts, at liquid nitrogen temperature. These emission bands have been attributed to the light-harvesting antenna LHC ($F_{680}$), to the antenna and the reaction center of PS II ($F_{685}$ and $F_{695}$) and also to the core antenna of PS I ($F_{695}$), and to the internal ($F_{720}$) and the peripheral ($F_{735}$) antennae of PS I. With green algae, only three main bands have been generally observed, at low temperature, near 685, 695 and 715–720 nm (see Refs. 4–6).

In the green alga *Chlamydomonas reinhardtii*, four main chlorophyll-protein complexes had been isolated and characterized, using lithium dodecylsulfate (LDS) polyacrylamide gel electrophoresis at 4°C: CP I related to PS I, CP II corresponding to the main light-harvesting antenna of both photosystems, CP III and CP IV related to PS II. CP I, CP III and CP IV contain only Chl *a*, while CP II contains both Chl *a* and chlorophyll *b* (Chl *b*) [7,8]. Two other minor complexes, which contain both Chl *a* and Chl *b*, have also been isolated; CP V, the function of which is unknown [8], and CP0 which probably corresponds to a part of the PS I antenna and which is responsible for a fluorescence emission near 707 nm at 77 K [9].

In order to characterize the relationships between low-temperature fluorescence emission bands, chlorophyll-protein complexes (as defined by their electrophoretic behaviour) and photochemical functions, it was of interest to examine the spectroscopic properties of the fluorescence emitted by eight mutants of *C. reinhardtii* which were deficient in one or several chlorophyll-protein complexes and showed impaired photochemical functions. These mutants have been previously isolated and characterized in our laboratory [10–13], except for one which originated from another laboratory [14,15]. The present work reports the results of such a study, and leads us to confirm the different correlations previously observed or proposed for higher plants and other algae. In addition, a new chlorophyll-protein complex, which was electrophoretically isolated and which appears to be related to the PS I antenna, is described.

## Materials and Methods

The characteristics of the wild type of *C. reinhardtii* and of the mutants *Pg* 27, *Fl* 5, *Fl* 39, *Fl* 50 and double mutants *Fl* 5 *Pg* 27, *Fl* 39 *Pg* 28, *Fl* 50 *Pg* 27 isolated in our laboratory, have been described in preceding papers [10,12,13,16–18]. The mutant *ac*-115, kept in stock cultures in our laboratory for about 15 years, was originally isolated and described by Dr. R.P. Levine (Harvard University, Cambridge) [14,15]. Algae were grown in light, in Tris-acetate-medium [19], as previously reported [17].

The Chl *a* and Chl *b* contents were measured according to Refs. 20 and 21. The photoreduction of DCIP was measured spectrophotometrically and the oxygen exchanges were measured by amperometry as previously described [10], using cells disrupted by mild sonication (70 W) for 15 s. For measurements of absorption spectra of cells at liquid nitrogen temperature, an Aminco DW2 spectrophotometer fitted with a special low-temperature attachment was used. The fluorescence emission spectra of pigment extracts, cells and excised polyacrylamide gel pieces containing isolated chlorophyll-protein complexes were measured at liquid nitrogen temperature, using an automated apparatus built in our laboratory. This apparatus permitted the use of 0.1 mm cuvettes and averaging of weak signals in order to minimize reabsorption artefacts.

For chlorophyll-protein analyses, either chloroplast fragments prepared as previously described [22] or Triton X-100-treated particles, enriched in both PS I and PS II, were used. These particles were prepared using the following procedure modified from Ref. 23: cells were disrupted by mild sonication (70 W) for 15 s, and treated once or twice with 0.45% (v/v) Triton X-100; cell debris was eliminated by centrifugation at $5000 \times g$ for 10 min and the Triton X-100-treated particles were obtained after centrifuging the supernatant at $20000 \times g$ for 30 min. Chloroplast fragments or Triton X-100-treated particles were analyzed, without prior lipid extraction or heating, by LDS-polyacrylamide gel electrophoresis according to Refs. 7 and 11. Depending on the chlorophyll-protein complexes, different solubilization mixtures were used: (a) 1 or 2% (v/v) LDS in 50 mM $Na_2CO_3$, 50 mM dithiothreitol and 10% glycerol, with a LDS/Chl $a$ + Chl $b$ ratio of 10 or 20, according to Ref. 7; or (b) 0.88% $n$-octyl-β-D-glucopyranoside with 0.22% sodium dodecylsulfate in 20 mM Pipes buffer (pH 6.6), 15 mM NaCl, 5 mM $MgCl_2$ and 100 mM sucrose, with an $n$-octyl-β-D-glucopyranoside/Chl $a$ + Chl $b$ ratio of 20, according to Ref. 24.

## Results

*Chlorophylls and photochemical activities*

The Chl $a$ and Chl $b$ contents and photochemical activities of the wild type and the different mutants are summarized in Table I. All the strains contained both Chl $a$ and Chl $b$, except for the mutant *Pg* 27 and the two double mutants *Fl* 5 *Pg* 27 and *Fl* 50 *Pg* 27 which had no Chl $b$, as indicated by the absence of a 654 nm peak on the fluorescence emission spectra of their ether-extracted pigments (Fig. 1), and the double mutant *Fl* 39 *Pg* 28 which showed only trace amounts of Chl $b$ as detected by a weak 77 K fluorescence emission at 654 nm from its pigment extract when excited at 470 nm. As calculated by Picaud and Dubertret [25], the sensitivity of the fluorescence emission method for detecting low amounts of Chl $b$ is very high (2 Chl $b$ for 1000 Chl $a$).

In the mutant *Pg* 27 both the photosystems were functional and despite its Chl $b$ deficiency this strain was able to carry out complete photosynthesis like the wild type. The mutant *Fl* 5 and the double mutant *Fl* 5 *Pg* 27 were unable to photoreduce methylviologen using $DCIPH_2$-ascorbate as an electron donor (Mehler reaction), indicating the absence of functional PS I. Both these strains, which performed poor DCIP photoreduction from $H_2O$ as electron donor, had functional PS II. On the contrary, the mutants *ac*-115, *Fl* 39, *Fl* 39 *Pg* 28, *Fl* 50 and *Fl* 50 *Pg* 27 showed normal PS I activity ($DCIPH_2$ → methyl viologen) but no PS II activity ($H_2O$ → DCIP). It was previously observed that *ac*-115, *Fl* 39 and *Fl* 39 *Pg* 28 never showed any PS II activity, whereas *Fl* 50 and *Fl* 50 *Pg* 27 were able to carry out weak DCIP photoreduction in the presence of diphenylcarbazide as an electron donor and showed a

TABLE I

CHLOROPHYLL CONTENTS AND PHOTOCHEMICAL ACTIVITIES OF THE WILD TYPE AND OF THE DIFFERENT MUTANTS OF *C. REINHARDTII*

(a) and (b) μg chlorophyll per mg dry matter. (b) tr., traces of Chl $b$ detectable only by 77 K fluorescence spectroscopy of the ether extract of pigments. (c) μmol of reduced DCIP $min^{-1}$ per mg of Chl $a$ + Chl $b$. Spectrophotometric measurement of the reduction of DCIP; algae concentration: 10 μg of Chl $a$ + Chl $b$ per ml (except for *Fl* 5 *Pg* 27: 5 μg per ml); red actinic light; $\lambda > 620$ nm, 300 $W \cdot m^{-2}$. (d) μmol of absorbed oxygen $min^{-1}$ per mg of Chl $a$ + Chl $b$. Amperometric measurement of oxygen absorption; algae concentration: 35 μg Chl $a$ + Chl $b$ per ml; red actinic light: $\lambda > 620$ nm, 440 $W \cdot m^{-2}$. (c) and (d) the cells which had been disrupted by mild sonication (70 W) for 15 s, were suspended in a mixture of 10 mM phosphate buffer (pH 7.5), 20 mM KCl, 2.5 mM $MgCl_2$, 2 mM $NH_4Cl$ and in addition: (3) 47 μM DCIP or (4) 0.25 mM DCIP, 15 mM sodium ascorbate, 10 μM DCMU, 0.1 mM methyl viologen and 1 mM $NaN_3$.

| Strains | Chlorophylls | | Activities | |
|---|---|---|---|---|
| | *a* | *b* | $H_2O$ → DCIP | $DCIPH_2$ → methyl viologen |
| | (a) | (b) | (c) | (d) |
| Wild type | 32.8 | 13.7 | 1.45 | 3.87 |
| *Pg* 27 | 14.3 | 0.0 | 1.08 | 4.27 |
| *Fl* 5 | 27.5 | 15.2 | 0.25 | 0.00 |
| *Fl* 5 *Pg* 27 | 9.5 | 0.0 | 0.40 | 0.00 |
| *Fl* 39 | 30.3 | 11.7 | 0.00 | 2.17 |
| *Fl* 39 *Pg* 28 | 21.8 | tr. | 0.00 | 4.78 |
| *Fl* 50 | 14.1 | 7.4 | 0.00 | 3.48 |
| *Fl* 50 *Pg* 27 | 13.8 | 0.0 | 0.00 | 5.67 |
| *ac*-115 | 31.7 | 15.9 | 0.00 | 3.37 |

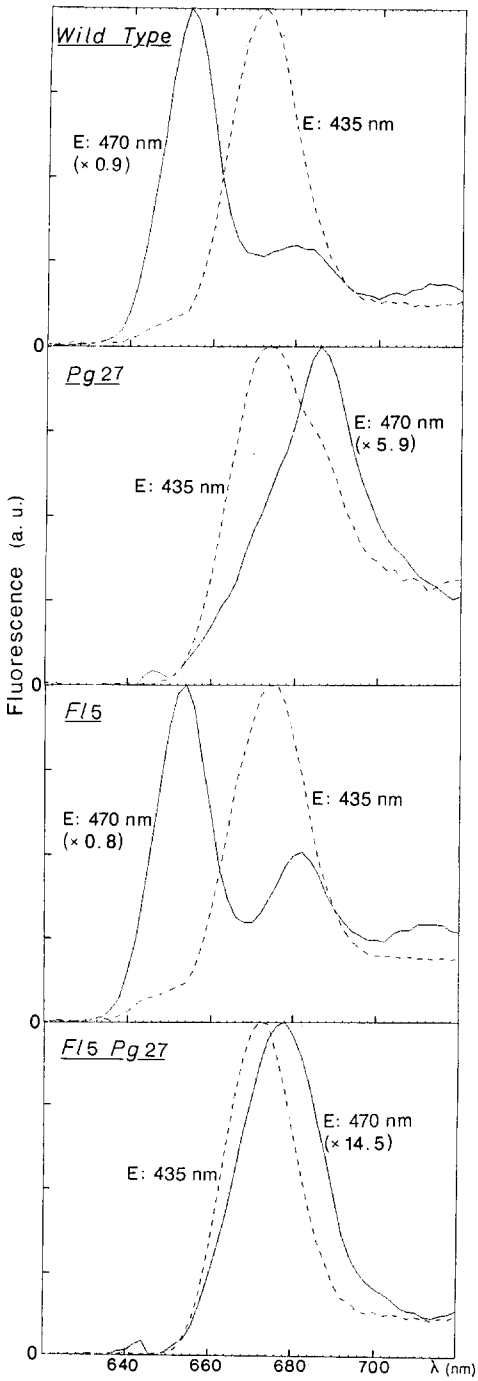


Fig. 1. Low-temperature emission spectra of ether extracts of pigments of the wild type and of the mutants *Pg* 27, *Fl* 5 and *Fl* 5 *Pg* 27 of *C. reinhardtii*. a.u., arbitrary units; *E*, excitation light wavelength. The extracts were frozen in 2 mm diameter tubes which were set against the front of the optical guide of the spectrofluorimeter and plunged into liquid nitrogen. Slit of the analytical monochromator, 2 nm. Note that for *Pg* 27 and *Fl* 5 *Pg* 27, the scales relative to $E = 470$ nm are 5.9 and 14.5 times, respectively, greater than those relative to $E = 435$ nm.

small degree of variable fluorescence [10,13]. A detailed study of fluorescence induction in *Fl* 50, at room temperature and at 77 K, indicated that in this mutant probably only 20% of the PS II reaction centers were functional (Briantais, J.-M., personal communication).

*Chlorophyll-protein complexes*

As previously described [11,13] and as summarized in Table II, the four Chl *b*-less mutants, *Pg* 27, *Fl* 5 *Pg* 27, *Fl* 39 *Pg* 28 and *Fl* 50 *Pg* 27, lacked the chlorophyll-protein complex CP II which corresponds to a main Chl *a* + Chl *b* antenna, equivalent to the LHC of higher plants [3,8]. Both the PS-I-deficient mutants *Fl* 5 and *Fl* 5 *Pg* 27 lacked the complex CP I which corresponds to the PS I reaction center and its core antenna [3,7]. On the other hand, the PS II-deficient mutants lacked the complexes CP III and CP IV, which correspond to the PS II reaction center and its antennae [7]. The mutants *ac*-115, *Fl* 39 and *Fl* 39 *Pg* 28 were totally devoid of CP III and CP IV, whereas *Fl* 50 and *Fl* 50 *Pg* 27 showed only traces of these complexes.

Another Chl *a* + Chl *b*-protein complex, CP 0 which corresponds to a part of the PS I antenna, has been observed with *C. reinhardtii* [9]. This complex was not seen on our previously published electrophoretograms and therefore we searched for it in all the different strains, using various solubilization mixtures for electrophoresis. The results of this analysis are indicated also in Table II and an example of an electrophoretogram is shown in Fig. 2. CP 0 was well isolated from chloroplast fragments when 0.88% *n*-octyl-β-D-glucopyranoside + 0.22% SDS were used as the solubilization mixture (Fig. 2, lanes e–h), whereas it was not always observed when 1 or 2% LDS was used (Fig. 2, lane c). This complex was present in the wild type and in the mutants *Fl* 5, *Fl* 39 and *ac*-115, but it was not found on the electrophoretograms concerning the four Chl *b*-deficient mutants *Pg* 27, *Fl* 5 *Pg* 27, *Fl* 39 *Pg* 28 and *Fl* 50 *Pg* 27. Surprisingly, on the electrophoretograms of the mutant *Fl* 50, which contains Chl *b* and shows normal PS I activity, only traces of CP 0 were observed.

Finally, a new chlorophyll-protein complex was clearly observed, as seen in Fig. 2, on electro-

TABLE II

CHLOROPHYLL-PROTEIN COMPLEXES OF THE WILD TYPE AND OF THE DIFFERENT MUTANTS OF *C. REINHARDTII*

As shown by polyacrylamide gel electrophoresis of chloroplast fragments or of Triton X-100-treated particles (see text). +, band present; −, band absent; tr., only traces were visible; n.a., not analyzed.

| Strains | Chlorophyll-protein complexes | | | | | |
|---|---|---|---|---|---|---|
| | CP 0 | CP 0a | CP I | CP II | CP III | CP IV |
| Wild type | + | + | + | + | + | + |
| *Pg* 27 | − | + | + | − | + | + |
| *Fl* 5 | + | + | − | + | + | + |
| *Fl* 5 *Pg* 27 | − | + | − | − | + | + |
| *Fl* 39 | + | + | + | + | − | − |
| *Fl* 39 *Pg* 28 | − | n.a. | + | − | − | − |
| *Fl* 50 | tr. | tr. | + | + | tr. | tr. |
| *Fl* 50 *Pg* 27 | − | n.a. | + | − | tr. | tr. |
| *ac*-115 | + | + | + | + | − | − |

phoretogram of Triton X-100-treated particles from the CP I-devoid mutants *Fl* 5 and *Fl* 5 *Pg* 27 and also from the PS II-lacking mutant *ac*-115, when 1 or 2% LDS was used in the solubilization medium. Fig. 2 also shows that this complex was not seen in non-Triton X-100-treated chloroplast fragments of the wild type. It was also observed with Triton X-100-treated particles of *ac*-115, that both Triton X-100 treatment and solubilization in 1 or 2% LDS were necessary for a good isolation of this new complex from CP I and CP 0. The location of this new complex close to CP I on the electrophoretograms and the fact that it was clearly observed in the mutant *ac*-115, totally devoid of PS II, infer that it is related to PS I. For this reason and taking into consideration the possible function of this complex (see Discussion), we designated it CP 0a. This complex CP 0a, the band of which appeared a little below the CP I band, had an apparent relative molecular mass which was slightly smaller than that of CP I and which appeared in the 78 kDa region, as estimated from Fig. 2. But this tentative value needs to be verified by more precise measurements. The fact that CP 0a was observed with the double mutant *Fl* 5 *Pg* 27, which was totally devoid of Chl *b*, indicates

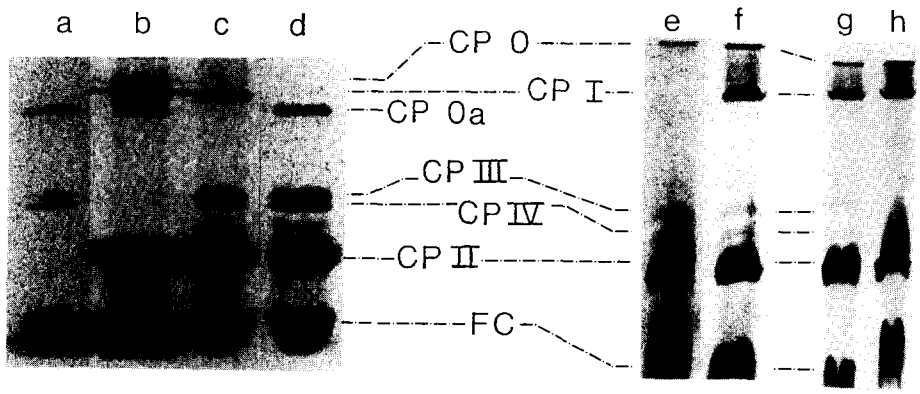


Fig. 2. Chlorophyll-protein complexes of the wild type (c, f, g) and of the mutants *Fl* 5 *Pg* 27 (a), *ac*-115 (b), *Fl* 5 (d, e) and *Fl* 39 (h) of *C. reinhardtii*. a–d: Triton X-100-treated particles (a, b, d) or chloroplast fragments (c) solubilized in 1% (a, c, d) or 2% (b) LDS, 50 mM $Na_2Co_3$, 50 mM dithiothreitol and 10% glycerol. e–h: chloroplast fragments solubilized in 0.88% *n*-octyl-β-D-glucopyranoside, 0.22% SDS, 20 mM Pipes buffer (pH 6.6), 15 mM NaCl, 5 mM $MgCl_2$ and 100 mM sucrose. LDS-polyacrylamide gel electrophoresis was then performed, at 4°C, as indicated in Refs. 11 and 13. Membrane concentrations: 14 μg (a) and 30 μg (b–h) of Chl *a* + Chl *b* per well. Unstained gel: all the bands were green pigmented. The photograph was taken through a blue filter (Wratten 38A). FC, free chlorophyll.

that it contains only Chl *a*. We searched for CP 0a with the different strains, except *Fl* 39 *Pg* 28 and *Fl* 50 *Pg* 27, and it was observed in all the cases. However, with the mutant *Fl* 50 only traces of CP 0a were present.

*Low-temperature absorption spectra of cells*

Fig. 3 shows absorption spectra of cells of different strains, measured at 77 K. On the classical spectrum of the wild type (see Refs. 1, 2, 26 and 27), the main peaks at 440 nm and 678 nm and the shoulder near 671 nm indicate the presence of several Chl *a* forms absorbing at different wavelengths in the red region, whereas the secondary peaks around 480 nm and at 652 nm indicate the presence of Chl *b*. On the spectrum of the Chl *b*-lacking mutant *Pg* 27 there was no peak in the 480 nm and 650 nm regions. In addition, there was no shoulder near 670 nm and the red peak was shifted to 679 nm indicating the absence of the main Chl *a* + Chl *b*-antenna (CP II) absorbing at relatively short wavelengths. The spectra of the mutants *Fl* 5 and *Fl* 5 *Pg* 27, both lacking PS I activity and the complex CP I, showed a single red peak which was shifted to shorter wavelengths (675–676 nm), indicating the absence of far red absorbing Chl *a* forms predominant in PS I. In addition, for *Fl* 5 *Pg* 27, the absence of Chl *b* was confirmed by the absence of peaks in the 480 nm and 650 nm regions. The spectra of

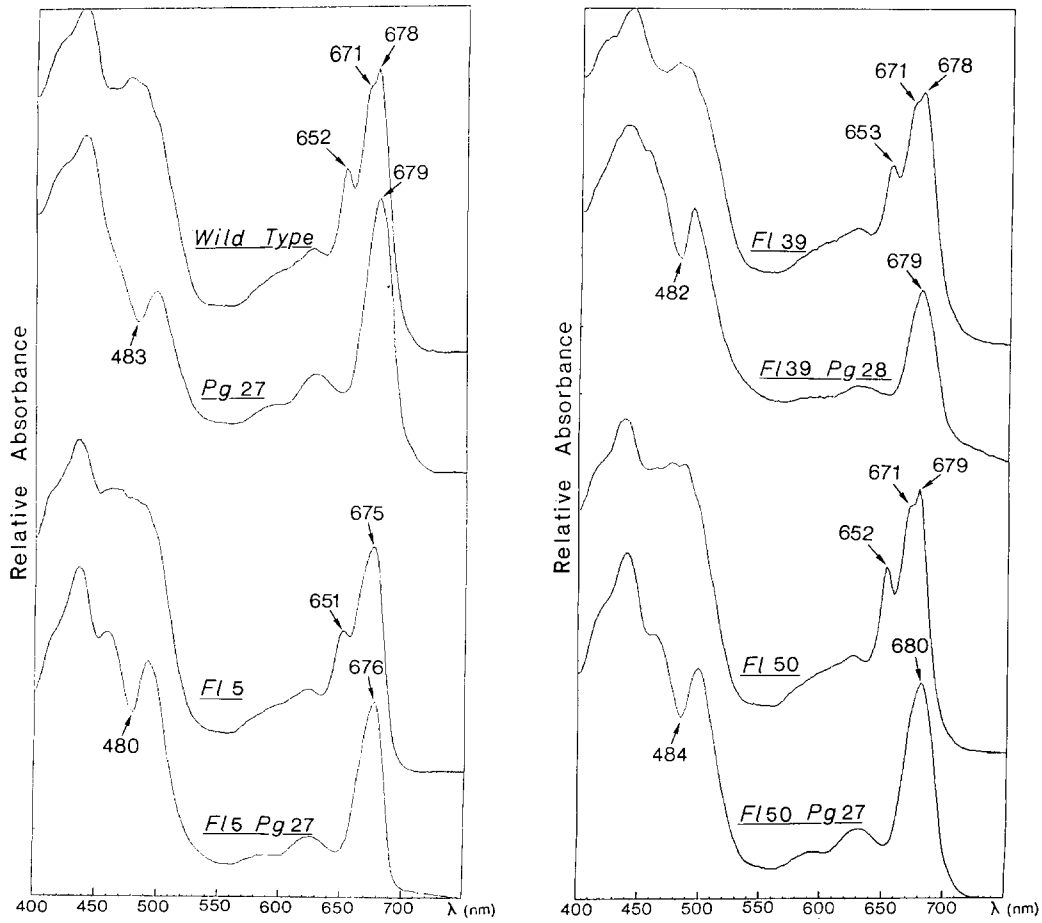


Fig. 3. Low-temperature absorption spectra of cells of the wild type and of the different mutants of *C. reinhardtii*. The cells (20, 30 or 40 μg of Chl *a* + Chl *b* per ml) were suspended in 66% glycerol then frozen in Bonner's cuvettes, the bottoms of which were plunged into liquid nitrogen in the spectrophotometer compartment. The spectra were normalized at their respective blue maxima in the 435–440 nm region. The numbers indicate the wavelengths of the peaks and shoulders.

both PS II-deficient mutants, *Fl* 39 and *Fl* 50, were similar to that of the wild type with peaks at 678 nm or 679 nm, 652–653 nm and in the 480 nm region, and shoulders near 671 nm, indicating the presence of Chl *a* and Chl *b* forms and the light-harvesting Chl *a* + Chl *b* antennae. On the spectra of the double mutants, *Fl* 39 *Pg* 28 and *Fl* 50 *Pg* 27, which were deficient both in PS II and in Chl *b*, a single red peak at 679 or 680 nm was observed showing the presence of Chl *a* forms absorbing at relatively long wavelengths (related to PS I). However, there were no peaks near 480 nm and 650 nm and no shoulder near 671 nm, indicating the absence of Chl *b* and the light-harvesting Chl *a* + Chl *b* antennae in these strains.

*Low-temperature fluorescence emission spectra of cells*

At room temperature, the fluorescence emission spectra of cells of all the strains were similar and showed a single peak in the 682–684 nm region (not shown). On the other hand, the spectra measured at 77 K pointed out significant differences between the strains as shown in Fig. 4. The spectrum of the wild type was comparable to the spectra observed with *C. reinhardtii* by several authors [12,25,26]. It showed a main peak at 686 nm, a shoulder near 696 nm and another broader peak around 713 nm. The emissions at 686 nm and 696 nm ($F_{686}$ and $F_{696}$) are attributed to PS II [5,6,28–30] and were observed as shoulders on the spectra of the three mutants *Pg* 27, *Fl* 5 and *Fl* 5 *Pg* 27 which had functional PS II. The shoulders near 686 nm were clearly visible on all the spectra, whereas the shoulders near 696 nm were always smaller than those at 686 nm and hardly discernible on the spectra of the mutants which had main peaks in the 700–710 nm region. On the contrary, $F_{686}$ and $F_{696}$ did not appear in the mutants, *Fl* 39 and *ac*-115, totally devoid in PS II. The emission around 713 nm corresponds to the PS-I-related long-wavelength emission, which was observed in the 710–720 nm region with the wild type and several mutants of *C. reinhardtii* [1,25,26,31] and is equivalent to the 735 nm emission of higher plants [5]. This emission (designated $F_{715}$) was observed as a main peak located between 712 and 717 nm on the spectra of the mutants *Pg* 27, *Fl* 39, *Fl* 39 *Pg* 28, *Fl* 50 *Pg* 27 and *ac*-115 which have functional PS I and the complex CP I, but it was absent on the spectra of the PS-I-deficient

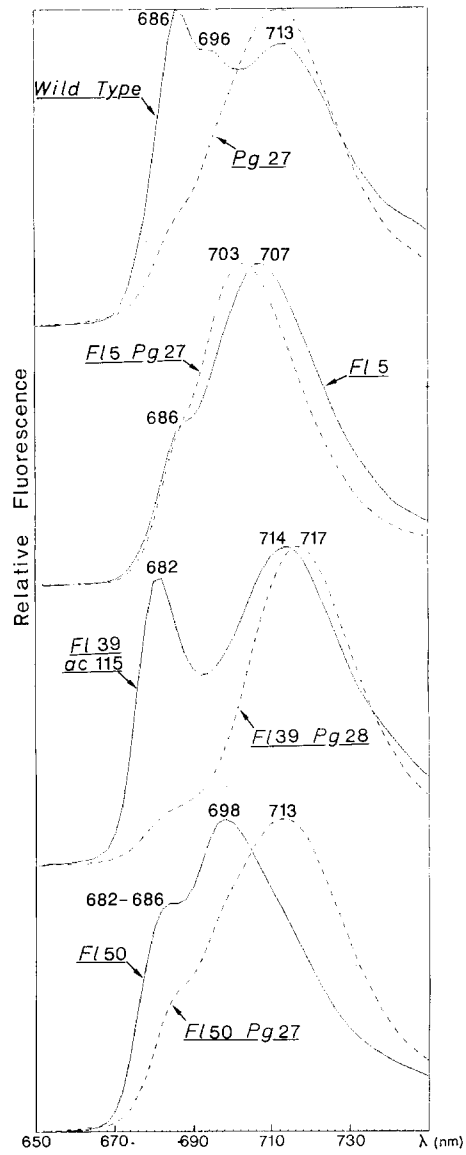


Fig. 4. Low-temperature fluorescence emission spectra of cells of the wild type and of the different mutants of *C. reinhardtii*. The cells (14, 22 or 50 μg of Chl *a* + Chl *b* per ml) were suspended in phosphate buffer (pH 7.5), then frozen in 0.1 mm thickness cuvettes which were set against the front 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 respective maxima. The numbers indicate the wavelengths of the peaks and shoulders.

mutants *Fl* 5 and *Fl* 5 *Pg* 27 and also, surprisingly, on the spectrum of the mutant *Fl* 50 which nevertheless shows important PS I activity (see below).

On the spectrum of the mutant *Pg* 27, $F_{715}$ was clearly the main emission and $F_{685}$ appeared as a shoulder, indicating that, in the absence of Chl *b* and of the light-harvesting complex CP II, PS I receives more light energy than PS II. The spectrum of the PS I lacking mutant *Fl* 5 was characterized by a shoulder near 687 nm and a main peak at 707 nm. The emission at 707 nm ($F_{707}$) was already observed with *Fl* 5 [12] and another PS-I-deficient mutant of *C. reinhardtii* [9] and it is attributed to the Chl *a* + Chl *b*-protein complex CP 0. On the spectrum of the double mutant *Fl* 5 *Pg* 27, which lacks Chl *b*, CP 0, CP I and CP II, $F_{707}$ did not appear but, in its place, a main emission at 703 nm was clearly observed along with a PS-II-related shoulder near 686 nm. This emission at 703 nm ($F_{703}$) could be due to a part of the PS I antenna, the fluorescence of which is not detectable when CP 0 and/or CP I were present.

The mutants *Fl* 39 and *ac*-115 did not show any emission in the 686 nm and 696 nm regions. Their spectra showed a peak at 680–682 nm ($F_{682}$) and a second peak around 714 nm due to PS I. A similar emission at 682 nm was also observed with another PS-II-deficient mutant of *C. reinhardtii*, F 34 [32]. $F_{682}$ could be attributed to the light-harvesting Chl *a* + Chl *b* antenna (CP II) which is unable to transmit its energy to PS II and consequently emits its own fluorescence [28,33]. The spectrum of the double mutant *Fl* 39 *Pg* 28 shows a main peak at 717 nm, typical of a PS I emission ($F_{715}$), and a shoulder in the 683 nm region. Like its parent *Fl* 39, *Fl* 39 *Pg* 28 does not show any PS II function and, on the electrophoretograms, it appears devoid of the complexes CP 0, CP II, CP III and CP IV. Nevertheless it contains some traces of Chl *b*, as shown by spectroscopy of pigment extracts (Table I). Therefore, it is possible that the weak emission near 683 nm corresponds to traces of CP II, only detectable by low-temperature fluorescence.

The spectrum of the mutant *Fl* 50, which shows a double shoulder near 682 nm and 686 nm and a main peak around 698 nm, is relatively difficult to explain. The double shoulder indicates the presence of light-harvesting antenna CP II and traces of CP III and CP IV found in this mutant which shows very low PS II-related photochemical activity. But, on the other hand, *Fl* 50 has the complex CP I and shows normal PS I activity and therefore it is surprising that the important PS I emission ($F_{715}$) did not appear on its spectrum, as on the spectra of the other PS-I-containing strains. Thus, it is probable that the energy transfer from CP II to CP I is impaired in *Fl* 50 and that the shift of the maximum of the broad emission band to a wavelength (698 nm) shorter than that of $F_{715}$ results from a very large contribution of the PS-II-related emissions $F_{686}$ and $F_{696}$, preferentially excited via CP II. Indeed, relatively high total fluorescence yields have been previously measured with *Fl* 50 [13]. In agreement with this explanation is the fact that the spectrum of the double mutant *Fl* 50 *Pg* 27, which differs from its parent *Fl* 50 by the lack of Chl *b* and complex CP II, shows a maximum around 713 nm, attributable to PS I. The *Fl* 50 *Pg* 27 spectrum also shows shoulders near 686 nm and 698 nm, confirming the presence of traces of CP III and CP IV in this mutant, as observed on the electrophoretograms.

*Low-temperature fluorescence emission spectra of isolated chlorophyll-protein complexes*

The 77 K fluorescence emission spectra of the different chlorophyll-protein complexes isolated by polyacrylamide gel electrophoresis and still bound to gel-support fragments are shown in Fig. 5. The maxima of the spectra of CP 0 (704 nm), of CP I (720 nm) and of CP II (680 nm) correspond to those previously measured with complexes from *C. reinhardtii* [9]. Isolated CP 0 and CP II show peaks at wavelengths a little shorter and CP I shows a peak at wavelengths a little longer than those of the emissions ($F_{707}$, $F_{682}$ and $F_{715}$) attributable to these complexes in whole cells, the spectra of which results from the contributions of several chlorophyll forms. CP III and CP IV show maxima at the same wavelength, 686 nm, which corresponds to the wavelength of the larger PS II-attributable emission ($F_{686}$) observed with whole cells. Several attempts to observe $F_{696}$ with chlorophyll-protein complexes isolated on polyacrylamide gels were unsuccessful. This emission, attri-

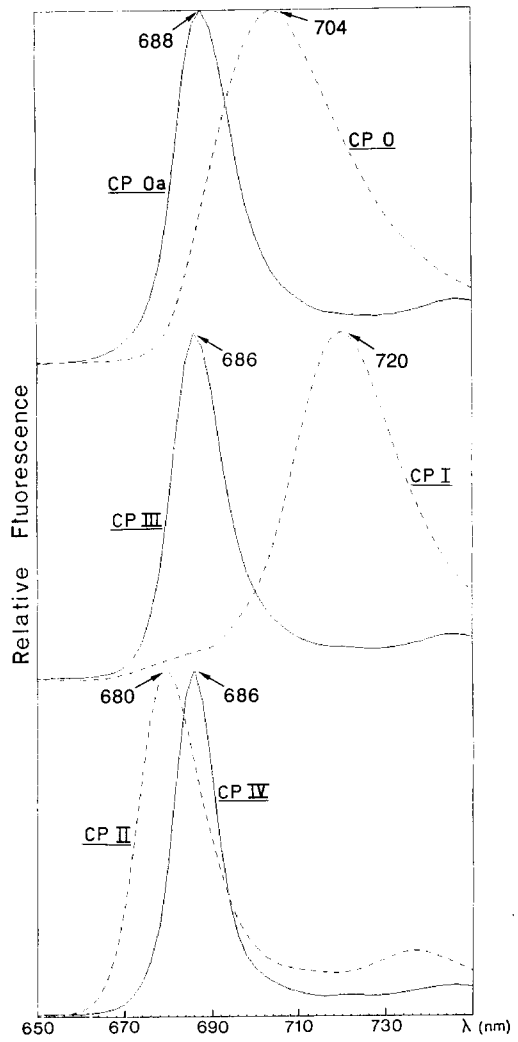


Fig. 5. Low-temperature fluorescence emission spectra of isolated chlorophyll-protein complexes of *C. reinhardtii*. Polyacrylamide-gel pieces (1.5 mm thickness), containing electrophoretically isolated chlorophyll-protein bands, were excised then frozen in a sample-holder which was set against the front 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 respective maxima. The numbers indicate the wavelengths of the peaks.

butable to the PS II reaction center, was very labile and often disappeared during the detergent-solubilization process used for Triton X-100-treated particles and for electrophoresis analysis (see Refs. 34 and 35).

The spectrum of the new chlorophyll-protein CP 0a shows an emission maximum at 688 nm, whereas an emission at 703 nm was observed with whole cells of the mutant *Fl* 5 *Pg* 27 in which CP 0a was the sole PS I antenna-related complex. It is possible that Triton X-100-treatment and/or LDS-solubilization, which were used for particles preparation and then for electrophoresis, induces a shift in the fluorescence emission to shorter wavelengths. A comparable surfactant-induced shift of 77 K fluorescence maximum was recently reported for the case of the fluorescence of PS-I-core complexes, which was measured with different thylakoid membranes and chlorophyll-protein complexes of higher plants [36]. It is also observed in this work, that the fluorescence yield of CP 0a was 1.7 times lower when excited at 475 nm than when excited at 435 nm. This observation confirms the absence of Chl *b* in CP 0a. Indeed, when Chl *b* was present, as in the case of CP 0 and CP II, the fluorescence yield was about two times higher under excitation at 475 nm than under excitation at 435 nm.

## Discussion

The results described here verify various relationships between electrophoretically defined chlorophyll-protein complexes and fluorescence emission bands observed at low temperature with cells of *C. reinhardtii*. A clear relationship appears between the fluorescence emissions $F_{686}$ and $F_{696}$, the chlorophyll-protein complexes CP III and CP IV and PS II. Indeed, $F_{686}$ and CP III and CP IV were observed with all the strains having functional PS II (wild type, *Pg* 27, *Fl* 5 and *Fl* 5 *Pg* 27). The mutants *Fl* 50 and *Fl* 50 *Pg* 27, which had impaired PS II function, even showed traces of CP III and CP IV and a clear $F_{686}$ emission. In addition, isolated complexes CP III and CP IV emitted fluorescence at 686 nm. $F_{696}$ was seen with the wild type and the mutant *Fl* 50 *Pg* 27 and was probably included in the main emission in the 700–710 nm region in the case of the other PS-II-containing strains. In contrast, the mutants *Fl* 39 and *ac*-115 which had no functional PS II did not show any emissions near 686 nm and near 696 nm and appeared devoid of CP III and CP IV. The spectrum of the double mutant *Fl* 39 *Pg* 28, also devoid of CP III and CP IV, showed only a small shoulder near 683 nm probably attributable

to traces of CP II. Nakatani et al. [37] have shown that, in PS II particles of spinach, $F_{695}$ was emitted by the chlorophyll-protein complex CP 47 which contains the PS II reaction center, whereas $F_{685}$ was emitted by a complex CP 43 which serves as an antenna for PS II. In *C. reinhardtii*, CP III corresponds to CP 47 and CP IV to CP 43. It has also been postulated that $F_{695}$ might originate directly from the PS II reaction center [38]. Similarly, a relationship between $F_{682}$ and the complex CP II was confirmed by comparing the spectra of the CP III and CP IV-deficient mutant *Fl* 39 and of the CP II, CP III and CP IV-deficient double mutant *Fl* 39 *Pg* 28. The large emission at 682 nm of the former mutant was reduced to a shoulder on the spectrum of the latter. In addition, isolated CP II emitted near 680 nm. The complex CP II, which contains Chl *a* and Chl *b* corresponds to the light-harvesting Chl *a* + Chl *b*-protein complex (LHC) of higher plants, which serves as an antenna for the two photosystems [33]. When energy transfer was good, as in cells of the wild type and of the mutant *Fl* 5, the fluorescence from CP II was not observed.

A close relationship between $F_{715}$, CP I and PS I was also confirmed. Both $F_{715}$ and CP I were absent in the case of the two mutants devoid of functional PS I, *Fl* 5 and *Fl* 5 *Pg* 27, and were present in the other strains, except for $F_{715}$ in the mutant *Fl* 50 the case of which will be discussed below. The fluorescence of isolated CP I was maximum around 720 nm. Another PS-I-related fluorescence emission, $F_{707}$, was clearly observed with the mutant *Fl* 5 and correlated with the presence of the complex CP 0, which emitted fluorescence near 704 nm when isolated on polyacrylamide gels. The complex CP 0, which contains both Chl *a* and Chl *b*, is considered to be part of the light-harvesting antenna of PS I which normally transmits light energy to CP I [9,31]. When CP I was absent, CP 0 emitted its own fluorescence at 707 nm.

A new type of fluorescence emission, *F*703, was observed with the double mutant *Fl* 5 *Pg* 27, which lacks Chl *b*, CP 0, CP I and CP II. This emission could be interpreted as originating from a Chl *a*-containing part of the PS I antenna distinct from CP 0 and CP I. The energy captured by this antenna would have been transmitted to CP I, when this latter complex was present. In the case of the mutant *Fl* 5 which lacks CP I but has CP 0, $F_{703}$ would have been masked by (or included in) the dominant $F_{707}$ emission. On the electrophoretograms of the same double mutant, *Fl* 5 *Pg* 27, only three spots of chlorophyll-protein complexes were observed: CP III and CP IV, which were related to PS II and the fluorescence of which is known, and the new complex CP 0a which appears to be related to PS I. Thus a correlation between CP 0a and $F_{703}$ is probable, despite the fact that CP 0a emits fluorescence at shorter wavelengths than $F_{703}$ when isolated on polyacrylamide gels after solubilization in LDS. Studying polypeptide patterns, chlorophyll-protein complexes and fluorescence emission spectra of a pale green photoautotrophic mutant, *y-1p*, of *C. reinhardtii*, Ish-Shalom and Ohad [31] have suggested that, in *C. reinhardtii*, energy from CP 0 is transferred to the core antenna of CP I via a connecting antenna which was supposed to be lacking in the mutant *y-1p*. However this antenna was not isolated as a particular chlorophyll-protein complex from the *y-1p* parent. The complex CP 0a observed in the present work could correspond to such a connecting antenna and would be the sole part of the PS I complex which remains in the double mutant *Fl* 5 *Pg* 27. Because CP 0a does not contain Chl *b*, we consider that it is probably distinct from CP 0 which contains both Chl *a* and Chl *b*, rather than being a part of this latter complex as proposed by Ish-Shalom and Ohad [31].

The energy transfer from CP II to PS I is probably impaired in the mutant *Fl* 50, as indicated by its fluorescence emission spectrum. On the other hand, only traces of CP 0 and of CP 0a were observed in this mutant which appeared deficient in PS I antennae. These observations indicate that CP 0 and CP 0a play an essential part in energy transfer from CP II to CP I. Some interaction between light-harvesting complex (CP II) and CP 0 has already been observed in *C. reinhardtii* by Wollman and Bennoun [9] who proposed that CP 0 could be directly involved in $Mg^{2+}$ regulation of energy distribution.

Fig. 6 illustrates the different energy transfer pathways and fluorescence emissions at 77 K deduced from the present results and the literature.

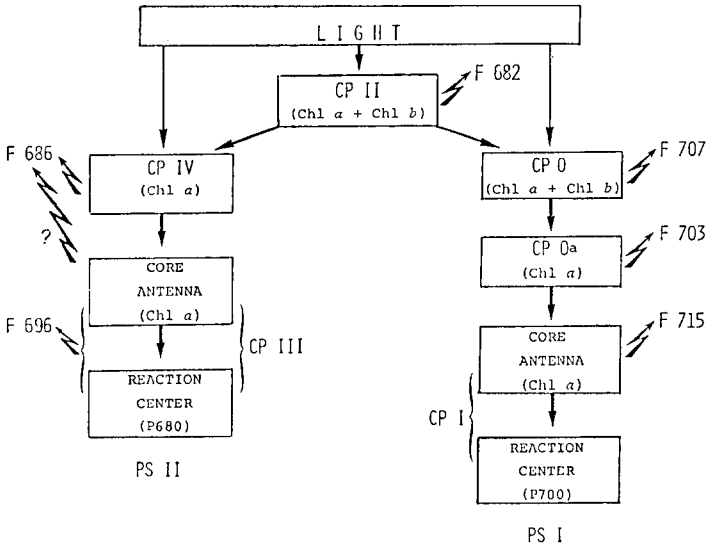


Fig. 6. Schematic representation of the light energy transfers through the different chlorophyll-protein complexes in *C. reinhardtii*. The straight arrows indicate the probable energy transfers which can be deducted from the present results and from the literature data; the zigzag arrows indicate the low-temperature fluorescence emissions from whole cells (see Discussion).

In the wild type, the light-harvesting complex CP II transmits energy to both photosystems. The transfer to PS II reaction centers occurs via CP IV, then via CP III which contains the core antenna and the reaction center (P-680). CP IV emits $F_{686}$ and CP III emits $F_{696}$ and perhaps also a part of $F_{686}$. The transfer from CP II to PS I occurs sequentially via CP 0, CP 0a and CP I which contains the core antenna and the reaction center (P-700). The core antenna emits $F_{715}$. In the mutants, when there is no CP III and no CP IV, CP II emits its own fluorescence $F_{682}$, as in the case of the strains *Fl* 39 and *ac*-115. When CP 0 and CP 0a are absent, no transfer from CP II to CP I occurs and CP II transmits only to PS II, as in the mutant *Fl* 50. When there is no CP I, CP 0 and also probably CP 0a emit their own fluorescence ($F_{707}$), as in the mutant *Fl* 5. When CP II is lacking, there is no common light-harvesting antenna and each photosystem captures light energy directly via its own antennae. In this situation the PS-II-related fluorescence emissions, when they occur, are lowered when compared to the PS-I-related emissions, as in the case of the mutant *Pg* 27 and the three double mutants. Finally, when there is no CP II, CP 0 and CP I, the fluorescence of CP 0a, $F_{703}$, is observed as in the case of the double mutant *Fl* 5 *Pg* 27.

In conclusion, this study of the fluorescence properties of a collection of mutants of *C. reinhardtii* permits us to confirm or to point out several relationships between different chlorophyll-protein complexes, as defined by their electrophoretic behaviour, and various low-temperature fluorescence emission bands from whole cells. A new Chl *a* protein complex, CP 0a, was observed. It appears to be related to the PS I antenna and is probably responsible for a low-temperature fluorescence emission at 703 nm. Further experiments will be carried out in order to complete the characterization of this complex CP 0a, especially with respect to its polypeptide composition.

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